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Virtual Population Analysis (VPA) Equations for Nonhomogeneous Populations, and a Family of Approximations Including Improvements on Pope's Cohort Analysis

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A set of "backward" virtual population analysis (VPA) equations relates catch (C_t) from continuous fishing between times t and $t + 1$ to population size (N_t, N_{t+1}) when a portion of the stock is unavailable to fishing. The usual VPA equations become a special case where the entire stock is available (i.e. the stock is homogeneous). A close approximation to the VPA equations is $N_t = N_{t+1} \exp(M) + C_t M/(1 - \exp(-M))$, which has properties similar to Pope's "cohort analysis" and is somewhat more accurate in the case of a continuous fishery, especially if the natural mortality rate (M) is large. Much closer simple approximations are possible if the seasonal pattern of catches is known.

Une série d'équations d'analyse de population virtuelle (APV) « à rebours » lie les prises (C_t) tirées de la pêche continue entre les temps t et $t + 1$ à la taille de la population (N_t, N_{t+1}) quand une partie du stock n'est pas disponible pour la pêche. Les équations habituelles d'APV deviennent un cas spécial là où le stock en entier est disponible (c.-à-d. que le stock est homogène). Une approximation étroite des équations d'APV est $N_t = N_{t+1} \exp(M) + C_t M/(1 - \exp(-M))$ qui a des propriétés semblables à l'analyse par cohortes de Pope et est un peu plus précise dans le cas d'une pêche continue, surtout si le taux de mortalité naturelle (M) est important. Des approximations simples beaucoup plus étroites sont possibles si l'on connaît la tendance saisonnière des prises.

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Virtual population analysis (VPA), also known generically as cohort analysis, has come to be the preeminent method of fish stock assessment since its appearance 20 yr ago (Gulland 1965; Murphy 1965). The method usually is used in the form of a "backwards solution" where historical abundances of a cohort are estimated on the basis of subsequent catches from a presumed homogeneous stock: all fish of a specified age are assumed to be equally susceptible to capture. The equations unfortunately do not allow convenient separation of the unknown quantities from the known and/or assumed quantities, so the solution must be obtained by numerical methods, e.g. Sims (1982), or by approximation, e.g. Pope's (1972) popular "cohort analysis." Computers have made numerical solution very easy, but there remain applications of approximation methods. For example, Pope's approximation has proved useful for statistical and mathematical analysis of the behavior of VPA, whereas the exact equations are intractable. In practice, good approximations and efficient algorithms remain useful, as computers may not conveniently be available in working group meetings, in field locations, and in some developing countries.

The following discussion provides two extensions to existing VPA methods. The first is a set of equations which include possible assumptions concerning homogeneity of cohorts, i.e. part of the cohort may not be available or vulnerable to fishing. These equations also provide the basis for an easy and rapid iterative backward solution of the VPA equations. The second is description of a family of approximation formulas which are similar in concept to Pope's cohort analysis.

VPA Equations for Nonhomogeneous Populations

Assume that the cohort consists of two segments, one fished and one unfished (cf. Widrig 1954). This division could arise under a variety of circumstances. For example, some members of the cohort may reside in habitats or regions which cannot be fished. Another possibility is differential vulnerability to the gear, depending on fish size or other attribute. The present treatment corresponds to the common model of "knife-edge" gear selection. Importantly, there must be no interchange between the two segments during the time interval. Let P be the age-specific fraction of the cohort which is subject to fishing at the beginning of a time interval. The remaining notation follows that of Ricker (1975), except that F refers to the instantaneous coefficient of fishing mortality *on the fishable segment of the cohort*. As usual, F and M are assumed to be independent, and M has the same value in both segments. Both of these assumptions are open to question (e.g. Lawless 1982, p. 491; Ricker 1976), but there seems to be no practical alternative

given the usual state of knowledge. All parameters are assumed to be constant within the time period, but may vary among ages or time periods. A full notation is useful for fishery analysis, but requires extensive use of subscripts denoting age and time; this notation can be developed by the reader for particular applications (Schnute 1985 provides a useful structure for this purpose). The present notation is simplified for ease of exposition, and considers a single cohort and a unit time period. The time subscript t is shown only where desirable for clarity. Under continuous fishing, the catch (C_t) for a unit time period beginning at time t, and ending at time $t + 1$, is given by

$$(1) \qquad C_t = N_t PFA/Z$$

where $Z = F + M$, and $A = 1 - \exp(-Z)$. The abundance at the beginning of the next time period is

$$(2) \qquad N_{t+1} = N_t P\exp(-Z) + N_t(1-P)\exp(-M)$$

where the two additive terms on the right side represent contributions from the fished and the unfished segments, respectively. This expression may be rearranged to give

$$(3) \qquad N_{t+1} = N_t P\,[\exp(-Z) - \exp(-M)] + N_t\exp(-M)$$

and equation (1) allows substitution of $C_t Z/FA = N_t P$, giving

$$(4) \qquad N_{t+1} = (N_t - C_t mZ/FA)\exp(-M)$$

where $m = 1 - \exp(-F)$. The backward solution therefore is

$$(5) \qquad N_t = N_{t+1}\exp(M) + C_t mZ/FA.$$

Equations (4) and (5) also can be derived from the equations describing the usually assumed homogeneous case. However, because P appears in equations (1) and (2), but not in (4) or (5), this derivation demonstrates that they have the somewhat surprising property of being true whether or not the homogeneity assumption ($P = 1$) is met, *provided that F is defined appropriately*. These equations also demonstrate that for a given catch and natural mortality rate, the four variables, N_t, N_{t+1}, P, and F, possess only two degrees of freedom among them. This means that specification of any two values should determine both remaining values, but this property may be of limited use as the basis for direct parameter estimation (e.g. for a given catch, F and P covary strongly, and estimation errors could easily lead to misinterpretation). In practice, one value is given; for example, in the case of a backwards solution, N_{t+1} may have been provided by an initial guess or by VPA of older aged catches. The second value is imposed by a simultaneous equation which may explicitly or implicitly fix P (see equations (6) and (7), respectively). Equation (5) is aesthetically attractive in that it clearly expresses the sense of "virtual population" as a modified sum of subsequent catches.

There are many possible equations which give F as a function of P, N_t, N_{t+1}, and M, depending on the assumed behavior and distribution of the stock and fishery. Differences among these equations become especially important when they are applied sequentially to link successive ages, as is the usual case in VPA. For example, equation (2) can be rearranged algebraically to give

$$(6) \qquad F = -\ln\left[\frac{(P-1)N_t + N_{t+1}\exp(M)}{PN_t}\right]$$

However, if P is treated as an independent population parameter, sequential use of this equation must be based on an assumed redistribution of all of the survivors at the end of each time period, so that the individuals in the fished and unfished segments have no differential tendency to remain in their previous states. Alternatively, if P can be estimated independently for individual ages and times, perhaps from surveys of habitat distribution or of size composition, equation (6) allows exploitation rates to be separated into component sources of variation: those associated with changes in P and those associated with changes in F on the available segment. This form of "separable VPA" (sensu Doubleday 1976) is being developed by Daniel Kimura (NMFS-NWAFC, BIN C15700, 7600 Sandpoint Way N.E., Seattle, WA 98115, USA, manuscript in revision). Of course, in the case of homogeneity ($P = 1$), equation (6) simplifies to

$$(7) \qquad F = \ln(N_t/N_{t+1}) - M$$

as was suggested by Pope (1972).

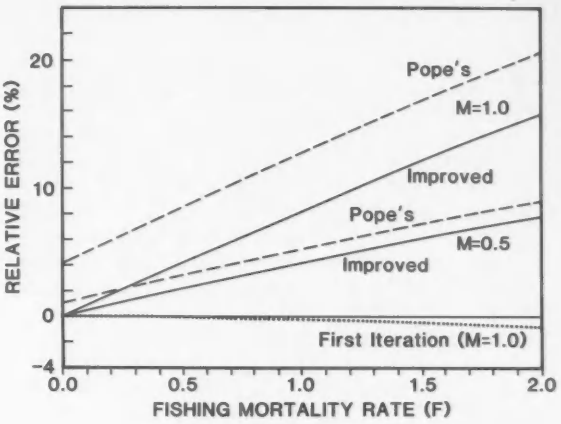

FIG. 1. Relative error (see text for definition) of Pope's and the new approximation for a homogeneous stock ($P = 1$) and two values of M. For comparison, the relative error of the result of the first iteration of the VPA algorithm is shown for $M = 1$, where relative error is defined as $((\hat{m}\hat{Z}/\hat{F}\hat{A})/(mZ/FA)) - 1$.

Equations (5) and (7) also provide a convenient rapid algorithm for solving the usual VPA equations for a homogeneous stock. The procedure is as follows: (1) given C_t, N_{t+1}, and M, use Pope's approximation or the improved approximation described below to obtain a first estimate of N_t; (2) use this value of N_t in equation (7) to obtain an estimate of F; (3) use this value of F in equation (5) to get an improved estimate of N_t; (4) repeat steps 2 and 3 until the desired level of precision is reached, judging by the change from the estimate in the previous iteration; and (5) repeat the procedure on the next younger age of the cohort, letting the previous N_t now be N_{t+1}, and go to step 1. The rate of convergence is very high (Fig. 1), approaching that of Newton's method as described by Sims (1982); two iterations often are sufficient. Also, this method does not converge to extraneous solutions.

A Family of Approximations

The independence of C_t in equation (5) indicates that there exists a family of VPA approximations of the form

$$(8) \qquad \hat{N}_t = N_{t+1}\exp(M) + C_t V(F, M, \ldots)$$

where $\hat{N}_t$ is an approximation of N_t and $V(F, M, \ldots)$ is a

function approximating the value of mZ/FA. These approximations are applicable to nonhomogeneous fisheries ($P \neq 1$) as well as the more commonly assumed homogeneous fisheries. In the case of nonhomogeneous fisheries, these equations need to be coupled with an equation such as (6).

Pope's (1972) cohort analysis is a member of this family of approximations, where $V = \exp(M/2)$:

$$\hat{N}_t = N_{t+1}\exp(M) + C_t\exp(M/2). \quad (9)$$

Another approximation is provided by a first-order Taylor series approximation of the exponentials in the left side of the expression

$$mn/A \approx FM/Z \quad (10)$$

where $n = 1-\exp(-M)$. Rearrangement of (10) gives

$$V = mZ/FA \approx Mn \quad (11)$$

which may be substituted into equation (8) to give

$$\hat{N}_t = N_{t+1}\exp(M) + C_tM/n. \quad (12)$$

The relative accuracy of approximations (9) and (12) can be expressed as the relative error in V, the coefficient of C_t; the remaining terms of the equations are identical and tend to reduce clarity of the comparisons. Also, because equations (9) and (12) are linear in C, errors in the coefficients propagate in a straightforward manner. Let the "relative error" (r_x) of an approximation, $\hat{X}$, be defined as $r_x = (\hat{X} - X)/X$, or equivalently, $r_x = (\hat{X}/X)-1$, where X is the true value. In the present case, the approximation is the coefficient applied to C_t in equations (9) and (12), and the true value is the coefficient mZ/FA in equation (5)). Thus, the relative error for Pope's approximation ($r_{(11)}$) is

$$r_{(9)} = \frac{\exp(M/2)}{mZ/FA} - 1 \quad (13)$$

where $\exp(M/2)$ is Pope's cohort analysis coefficient of C_t. Similarly, the relative error of the new approximation in equation (12) is

$$r_{(12)} = \frac{M/n}{mZ/FA} - 1. \quad (14)$$

Relative errors for these two approximations are compared in Fig. 1; generally, the differences are not large. The relative error of the new approximation in equation (12) is slightly but consistently smaller than that of Pope's approximation. The new approximation becomes considerably better than Pope's as M becomes large, thus extending the range of mortality rates under which approximation is useful. Taylor series expansions of the relative errors, equations (13) and (14), clarify the nature of the difference between the two approximations. If we discard terms higher than quadratic, the relative error for Pope's approximation is $(2FM + M^2)/24$, whereas that of the new approximation is $(2FM)/24$. The constant factor of $M^2/24$ in Pope's relative error explains both its nonzero intercept at $F = 0$ and the parallel appearance of the plots in Fig. 1.

Pope's approximation is equivalent to assuming that the entire catch is taken instantaneously at midyear. The new approximation does not have a corresponding convenient interpretation. A tentative interpretation may be derived from the relationship $\bar{N} = N_0n/M$, which describes average abundance ($\bar{N}$) over a unit time interval versus initial abundance (N_0), if only natural mortality is acting. Thus, the quantity M/n used in the new approximation is equal to $N_0/\bar{N}$ if there is only natural mortality. Paradoxically, this means that the new approximation assumes the fishery's catch to be a continuous sampling of natural deaths in the absence of a fishery. This somewhat strange interpretation is consistent with the fact that the new approximation is identical to the expression which results from taking the limit of equation (5) for a given catch as $F \rightarrow 0$.

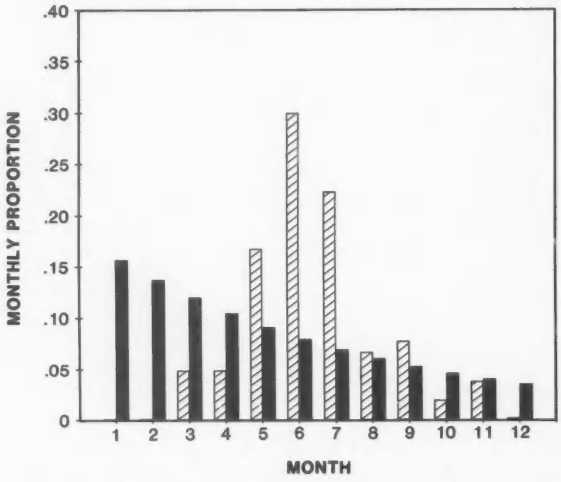

FIG. 2. Comparison of monthly catch distribution under the assumption of constant F and that from a seasonal fishery (the California catch of mackerel in 1941–42, Anonymous 1944).

VPA Approximation for Seasonal Fisheries

The usual assumption in VPA is that F is constant over the time interval under consideration. This assumption requires that the catch decline exponentially over that interval. Many, if not most, fisheries are seasonal, so the pattern of catches over the year or season does not conform to this assumption (Fig. 2). A standard practice in these cases is to divide the fishing year into shorter time periods such as quarters or months, within which F is more nearly constant.

If the seasonality of the fishery is predictable, a useful approximation can be developed as follows. If the exact time (t_i) of each catch (C_i) were known, VPA equation (8) would reduce to

$$N_t = N_{t+1}\exp(M) + \sum_i C_i\exp((t_i - t_0)M) \quad (15)$$

where t_0 is the beginning of the fishing year. Equivalently, if the proportion (p_i) of the total catch (C_t) taken at time t_i is known, we have

$$N_t = N_{t+1}\exp(M) + C_t\sum_i p_i\exp((t_i - t_0)M). \quad (16)$$

Equation (16) has the same form as equation (8), demonstrating that seasonal fisheries can be treated by the same family of approximations. Moreover, equation (16) requires information only on M and the temporal distribution of catches, making function V_{seas}, and hence the VPA, independent of estimated F.

In practice, the value of V_{seas} for a seasonal fishery can be

obtained from sequential VPA of the typical monthly proportions of the total annual catch (see example below). By setting N_{t+1} to zero, V_{seas} is given directly by the virtual population of the p_i values at time t_0, this being equivalent to the summation in equation (16).

Application of Approximations

Parrish and MacCall (1978) gave VPA estimates for mackerel (*Scomber japonicus*), based on annual catch-at-age data, $M = 0.5$, and assumed constant F within the fishing season. Estimated F for fish aged 4 and older in the 1941–42 fishing year (May through April) was 1.115, giving a V_{annual} of $mZ/FA = 1.2152$. The monthly proportions of the total unaged annual catch are based on Anonymous (1944) and are shown in Fig. 2. These monthly catches are in weight rather than numbers, causing some error due to individual growth during the year. A value of V_{seas} can be approximated by letting $V_{month} = (M/12)/(1-\exp(-M/12))$. The annual value of V based on the seasonal catch distribution is approximately

$$(17) \quad V_{seas} = \sum_{i=1}^{12} p_i V_{month} \exp((i-1)M/12)$$

giving $V_{seas} = 1.2851$. Thus, the relative error of Parrish and MacCall's annually based VPA is approximately −5.4% for this year, indicating a moderate underestimate of abundance, given the assumptions. Interestingly, the new VPA approximation in equation (12) gives $V_{new} = 1.2707$, and Pope's approximation gives a nearly exact value of $V_{Pope} = 1.2840$. Pope's approximation does well because in this case the actual catch was indeed taken very near midyear. Of course, other seasonal distributions may produce values of V_{seas} ranging from 1.0 (all the catch taken at year's end) to $\exp(M)$ if all of the catch is taken at the beginning of the year.

Differences in VPA estimates of abundance due to various homogeneity assumptions and/or approximations often fall within the variability due to imprecision in the value of the natural mortality rate, M. For example, given $M = 0.5$ and constant $F = 0.5$, Pope's approximation using $M = 0.48$ would have the same relative error as the new approximation using $M = 0.5$. The difference becomes larger for large M: given $M = 1.0$ and constant $F = 1.0$, Pope's approximation would require $M = 0.92$ to produce a relative error equal to that of the new approximation. Violations of the assumption of constant F over the time period can produce somewhat larger errors. The relative error of Parrish and MacCall's annual VPA in the preceding example is roughly equal to the relative error which would have occurred if they had used a seasonally corrected VPA with $M = 0.39$, which is 22% smaller than assumed M.

It can be argued that the choice of approximations is inconsequential because imprecision associated with alternative VPA approximations and assumptions is smaller than that associated with imprecision in assumed M. A counterargument is that errors due to approximations or assumptions are systematic biases and should be recognized as such. If a value of M is stated, VPA using that M should as nearly as possible reflect that value, rather than some systematically different effective value arising from the properties of an unnecessary assumption or approximation.

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Benthic Macrofaunal Production in the Bay of Fundy and the Possible Effects of a Tidal Power Barrage at Economy Point – Cape Tenny

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The distribution of macrofaunal productivity in the Bay of Fundy, inclusive of the upper Bay (Chignecto Bay and Minas basin) is presented. The data used in mapping are based on wet biomass from 266, 0.1- and 0.5-m^2 grab samples, converted to annual productivity using a lifespan/annual turnover regression. Macrofaunal production of the subtidal area (11 149 km^2) of the whole Bay of Fundy is estimated to be 212×10^4 t wet biomass$\cdot yr^{-1}$ equivalent to 190 g wet wt$\cdot m^{-2} \cdot yr^{-1}$. Production in the intertidal area (1437 km^2), by contrast, is estimated to be 13×10^4 t wet biomass$\cdot yr^{-1}$ or 92 g wet$\cdot m^{-2} \cdot yr^{-1}$. Possible qualitative macrofaunal changes resulting from building a tidal barrage between Economy Point and Cape Tenny are suggested on the basis of predicted physical changes, particularly tidal current speed, sediment type, and sediment bed stress. The only quantitative prediction possible with our biological data is that suspension-feeding animal production will be 17% less in the lower Bay of Fundy following barrage construction. This reduction is not significant at the 95% confidence level.

Les auteurs présentent la distribution de la productivité macrofaunique dans la baie de Fundy, y compris la baie de Chignecto et le bassin de Minas. Les données utilisées sont basées sur une biomasse humide de 266 prélevements de 0,1 et de 0,5 m^2 transformés en productivité annuelle par régression du renouvellement annuel. La production de la macrofaune de la zone infratidale (11 149 km^2) de toute la baie de Fundy est estimée à 212×10^4 t de biomasse humide par année, ce qui équivaut à 190 g$\cdot m^{-2} \cdot a^{-1}$ (poids humide). La production de la zone intertidale (1 437 km^2) est, quant à elle, estimée à 13×10^4 t de biomasse humide par année, soit 92 g$\cdot m^{-2} \cdot a^{-1}$ (poids humide). Les auteurs traitent des modifications qualitatives possibles de la macrofaune qui pourraient résulter de la construction d'un barrage marémoteur entre la pointe Economy et le cap Tenny en se basant sur les modifications physiques prévues d'une telle construction, notamment la vitesse du courant de marée, le type de sédiments et la contrainte sédimentaire sur le lit. La seule prévision quantitative pouvant être faite à partir des données biologiques est que la production des animaux se nourrissant de particules en suspension sera inférieure de 17 % dans la partie inférieure de la baie suite à la construction du barrage. Cette réduction n'est cependant pas significative au niveau de confiance de 95 %.

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Since the 1920's a series of proposals have been made to dam parts of the Bay of Fundy in order to utilize some of the large tidal energy resources available to drive turbines and thus produce electricity. Civil engineering details of alternative barrage sites were presented at a meeting held in Wolfville in 1976 (Daborn 1977). This meeting resulted in several research projects by the local scientific community to help predict the effects of a large tidal barrage on the physical and biological characteristics of the Bay.

Recent work concerning the Bay of Fundy has increased our knowledge of surficial sediment distribution (Fader et al. 1977), tidal current predictions (Greenberg 1976, 1979), benthic conditions (Peer et al. 1980; Wildish and Peer 1983), and annual production estimates of several dominant benthic animals (Wildish 1984). In addition, an empirical relationship was established between horse mussel (*Modiolus modiolus*) production and current speed in the lower Bay (Wildish and Peer 1983).

The major purpose of this paper is to present a macrobenthic productivity chart of the whole Bay of Fundy, including previously published information on the lower Bay (Wildish and Peer 1983) before tidal barrage building takes place.

Secondly, a first attempt is made here to predict the effect on the macrobenthos of the Bay of Fundy if one particular site, the

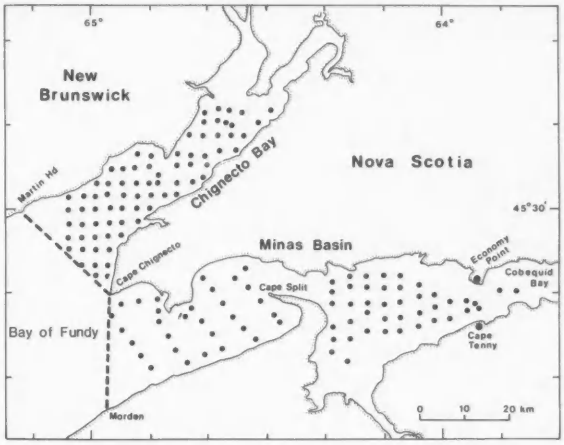

FIG. 1. Stations sampled in the Upper Bay of Fundy: 77 in Chignecto Bay in 1978 and 73 in Minas Basin in 1979.

B9 barrage (see Clark 1977; Rowsell 1977; Baker 1984) located in Cobequid Bay, was built from Economy Point to Cape Tenny.

Materials and Methods

Benthic sampling was carried out on three cruises as follows: lower Bay of Fundy in May 1978 from the *J. L. Hart* using a 0.1-m^2 Hunter and Simpson grab (Hunter and Simpson 1976); Chignecto Bay in August 1978 from the *CSS Dawson* using a 0.1-m^2 Van Veen grab; and Minas Basin in August 1979 from the *CSS Dawson* generally using a 0.5-m^2 Van Veen grab but occasionally using both types of 0.1-m^2 grabs for comparative purposes. A full 0.1-m^2 sampler contained 10–16 L, whereas a full 0.5-m^2 sampler contained 70–80 L, of sediment slurry. Generally, two 0.1-m^2 samples were taken at each station and combined for analysis. However, in Chignecto Bay and for the large grabs taken in the Minas Basin, only a single grab sample was obtained at each station. Location details for the lower Bay sampling are given in Wildish and Peer (1983); the Chignecto Bay and Minas Basin stations are shown in Fig. 1 (coordinates are given in Wildish et al. 1983). Sampling depths ranged from 15 to 208 m with the deeper stations being at the mouth of the lower Bay.

Sediment from each grab was sieved on deck with running seawater through a sieve series, the finest having a square mesh size of 0.8 mm. Retained macrofauna were killed and preserved in 10% formalin in seawater. In the laboratory, organisms were sorted into family, genera, or species groups in dilute alcohol, weighed to the nearest milligram, and counted before taxonomic verification by the Atlantic Reference Centre, St. Andrews. Analysis of macrofaunal data was similar to that in Wildish and Peer (1983). Density (number per square metre) and biomass (grams wet weight per square metre) were calculated from the grab sampling area and expressed per square metre. No attempt was made to correct for the volume of sediment sieved or to allow for changes in fresh to preserved wet weight of the macrofauna. These systematic errors were considered to be less important than those associated with benthic grab sampling itself. Secondary production (grams wet weight per square metre per year) was determined by multiplying the wet biomass value by the $P:B$ value and summing for each species present at the station. $P:B$ ratios for each species in our study were derived from the inverse logarithmic relationship of lifespan (x, years) derived from literature sources and $P:B$ (y) (Robertson 1979):

$$\log_{10} y = 0.66 - 0.726 \log_{10} x,$$

Each species was categorized into one of five trophic groups (algal scraping, carnivore, omnivore, suspension-feeding, or deposit-feeding) as in Wildish and Peer (1983). Productivity charts were plotted by hand, and where necessary, estimates were made to interpolate between points. A parametric test was made of the null hypothesis that a sample production mean for a specified geographical area was from the same population in the Bay. We also tested the hypothesis that the $\log_{10}$ suspension-feeder production was a linear function of the current speed at the same station.

The spatial limits of the lower and upper Bay used in estimating productivity, inclusive of Chignecto Bay and Minas Basin, are shown in Fig. 1 and 2. Areas were estimated directly from charts by planimetry. Surficial sediment conditions were also available from charts, but for the lower Bay of Fundy only (Fader et al. 1977). Tidal energy data were available for each of the grid boxes in the Greenberg (1979) model of the whole Bay of Fundy – Gulf of Maine. This model includes the three principal semidirunal components, M_2, N_2, and S_2, for the year 1978 and two principal diurnal components, O_1 and K_1. Hourly predictions and model runs incorporating various tidal barrages, including the B9 barrage, were made from this model (Greenberg 1979). Output from the model includes maximum tidal current speed, V_{max} (centimetres per second), and average tidal current speed, V_{avg} (centimetres per second), integrated throughout the water column and available for many of the biological sampling stations. Bed shear stress for each station has been calculated from V_{avg} rather than from V_{max} (Warwick and Uncles 1980; Wildish and Peer 1983) because the latter may overestimate average bed stresses in high tidal energy environments. Average bed shear stress, r_0 (dynes per square centimetre), is calculated as

$$r_0 = \gamma p V_{avg}^2$$

where $\gamma = 3 \times 10^{-3}$ is a dimensionless friction coefficient and $p = 1.03$ g·cm^{-3} is the density of seawater, both treated as constants.

Results

Macrobenthic Species and Productivity, 1978–79

A complete species list was obtained from 248 different stations within the Bay of Fundy and includes 264 taxa identified to species, 55 to genera, and 25 assigned only to a higher taxon.[1] The trophic type category for each species is that which is considered to be the most important feeding method. For some species, e.g. *Haploops fundiensis*, which may change from suspension-feeding to deposit-feeding (Enequist 1950) as the current conditions change during the tidal cycle, it is difficult to specify the appropriate trophic type.

All of the 344 identified taxa were used for production esti-

[1]The complete species list is available, at a nominal cost, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ontario K1A 0S2.

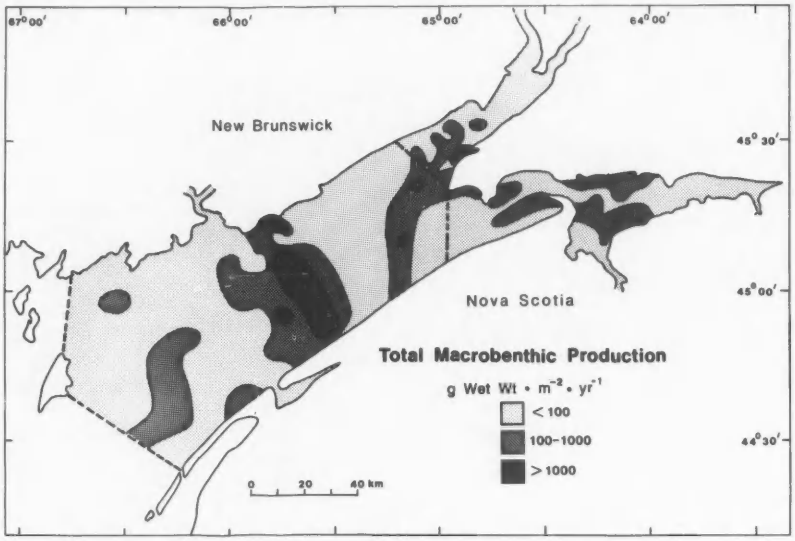

FIG. 2. Bay of Fundy showing the total benthic macrofaunal production for 1978–79 with isolines drawn connecting >1000, 100–1000, and <100 g wet wt·m^{-2}·yr^{-1}.

TABLE 1. Regression constants for the relationship between the $\log_{10}$ suspension-feeder production, x (g wet wt·m^{-2}·yr^{-1}), and the average current speed, y (cm·s^{-1}), in the equation $\log_{10} x = a + by$.

Location	a	b	r	N
Lower Bay	−1.38	0.06	0.84	33
Minas	1.42	0.01	0.36	34
Chignecto	−0.71	0.02	0.25	35

mation. The chart showing total macrofaunal production throughout the Bay (Fig. 2) indicates four general areas with production values in excess of 1000 g wet wt·m^2·yr^{-1}. Two of these occur in the lower Bay, with the second extending into Chignecto Bay and two in Minas Basin. The centres of high production, mainly represented by the horse mussel, are separated by ~35 km and this approximately equals two excursion distances of the tides of this macrotidal estuary. However, high production seems to be limited to the less than 50 m depth contour; production is much lower in the deeper (up to 200 m) mouth of the Bay of Fundy.

A characteristic feature of the Minas Basin sublittoral macrofauna is the presence of species adapted to withstand very high current speeds by attaching firmly to a hard substrate, e.g. *Balanus crenatus, Crepidula fornicata, Polydora* sp., and small forms of an actinarian, all of which are suspension-feeders. Characteristic deposit-feeders of Cobequid Bay and Minas Basin are the polychaetes *Eulalia viridis, Euclymene zonalis, Polycirrus* sp., and the sipunculid *Golfingia* sp.

Tidal Currents and Suspension-feeding Macrobenthic Fauna

Tidal current velocities increase landwards in the Bay of Fundy. In Minas Basin near Cape Split, $V_{max} \geq 400$ cm·s^{-1}, which is the highest speed achieved anywhere in the Bay.

Previous analysis of the lower Bay data (Wildish and Peer 1983) indicated a strong positive relationship between current speed (≈ seston supply rates) and the $\log_{10}$ of suspension-feeder production. However, similar data for the upper Bay (Table 1) show that this relationship is not significant at $p <$ 0.05 either for Minas Basin or Chignecto Bay.

Trophic Groups and Benthic Productivity

Suspension-feeding animals were responsible for the highest annual production rates, with values in excess of 1000 g wet wt·m^{-2}·yr^{-1}. The distribution of suspension-feeding animal production follows closely that of total secondary production shown in Fig. 2 because it makes up over 80% of the total. High suspension-feeder production occurs in the mouth of Chignecto Bay and in Minas Basin (Fig. 3). In addition, an area of lesser production, inclusive of horse mussels, extends seaward on either side of the tidally scoured channel near Cape Split. This suggests an impoverishing effect caused by the extremely high tidal streams in the central channel of this area. As in the lower Bay, much of this suspension-feeder production represents an energy sink because horse mussels are relatively long-lived (Depository of Unpublished Data, CISTI). However, there is an annual release of gametes to the plankton, representing a substantial, but as yet unquantified, addition of energy to that part of the ecosystem. By contrast, deposit-feeding animal production is much lower (Fig. 4).

Calculations based on mean production in Chignecto Bay and Minas Basin (Fig. 3 and 4) are used to determine total secondary production (Tables 2 and 3). Total macrobenthic production in Minas is approximately twice that of Chignecto, proportional to their respective areas. Intertidal production is relatively important in both arms of the upper Bay, accounting for 13–19% of total benthic production. This compares with less than 3% in the lower Bay (Wildish and Peer 1983). Whereas production of suspension-feeders makes up over 80% of the macrofaunal total in Minas (Table 2), it is less than 70% in Chignecto Bay (Table 3). Concomitantly, the importance of deposit-feeders is greater in Chignecto (>10% of total production) than in Minas where <1% is deposit-feeding animal production.

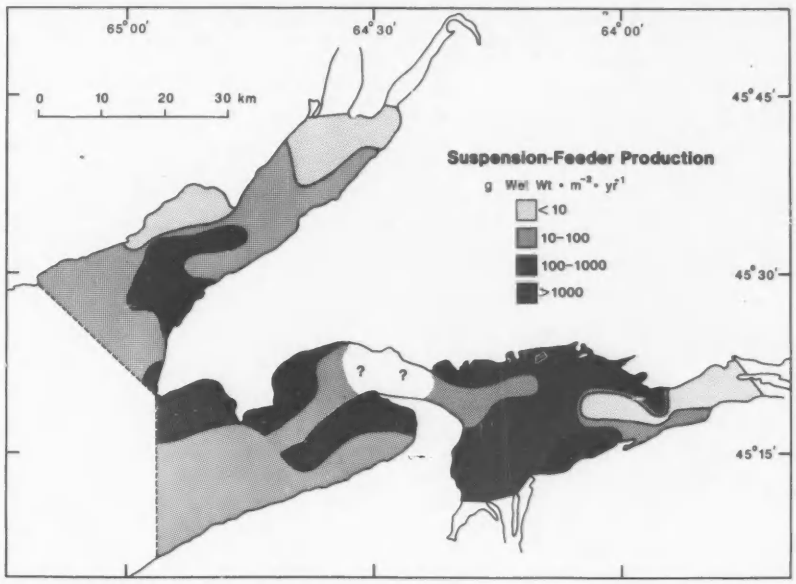

FIG. 3. Upper Bay of Fundy showing suspension-feeding production with isolines drawn connecting >1000, 100–1000, 10–100, and <10 g wet wt·m^{-2}·yr^{-1}.

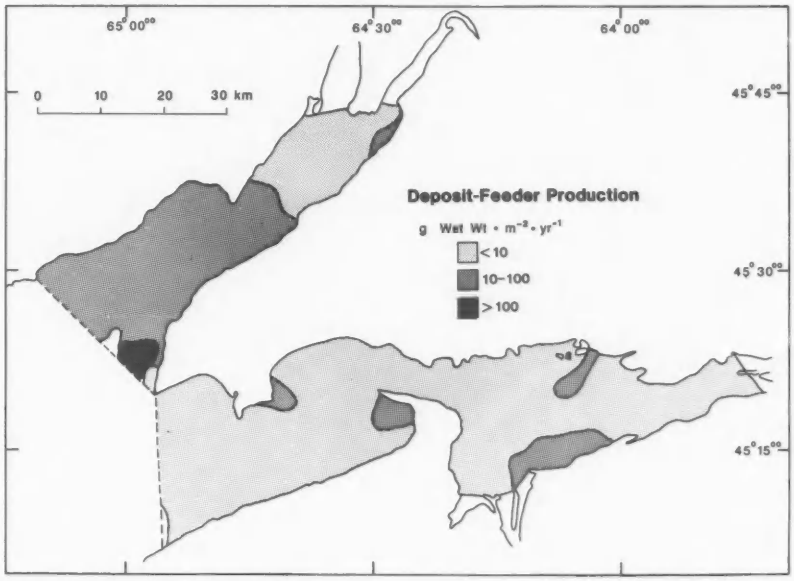

FIG. 4. Upper Bay of Fundy showing deposit-feeding animal production with isolines drawn connecting >100, 10–100, and <10 g wet wt·m^{-2}·yr^{-1}.

Predicted Effects of a Barrage at Economy Point – Cape Tenny (B9)

The most obvious physical change expected from the presence of the B9 barrage would occur within Minas Basin involving slower currents flowing into, and out if, Cobequid Bay (Fig. 5). We suggest that there would be a reduction in production of populations of suspension feeding macrofauna, primarily horse mussels, in Cobequid Bay. Because there was no significant relationship between current speed and suspension-feeding animal production in the Upper Bay of Fundy (Table 1), we are unable to predict the production lost due to the reduced current speed in this area. For the lower Bay where this relationship has been found to be significant, it is possible to predict from the tidal current patterns (Greenberg 1979) what the suspension-feeding animal production should be, with or without the barrage in place (Table 4). This prediction is based on the 56 stations for which we have complete tidal current and

TABLE 2. Secondary benthic production of macrofauna in units of wet biomass for Minas Basin, excluding the Avon River and Upper Minas (estimated on the basis of the geographic distribution shown in Fig. 3 and 4 and a total area of 2010 km^2).

Trophic group	Production unit (g wet wt·m^{-2}·yr^{-1})	Observed mean for each production unit (t·km^{-2}·yr^{-1})	Area of production unit (km^2)	Annual production (t × 10^4)	Percentage ot total production	Total (t × 10^4)
Suspension feeders	0–10	0.40	116	0.01		
	10–100	24.58	657	1.61		
	100–1000	383.28	664	25.45		
	>1000	2191.00	35	7.67	83.55	34.74
Deposit feeders	<10	2.52	1352	0.34		
	>10	18.77	120	0.02	0.87	0.36
Others	—	7.44	1472	1.10	2.65	1.10
Mixed intertidal	—	100.00[a]	538	5.38	12.94	5.38
					100	= 41.58

[a]Estimated.

TABLE 3. Secondary benthic production of macrofauna in units of wet biomass for Chignecto Bay, excluding the Shepody and Cumberland basins (estimated on the basis of the geographic distribution shown in Fig. 3 and 4 and a total area of 1099 km^2).

Trophic group	Production unit (g wet wt·m^{-2}·yr^{-1})	Observed mean for each production unit (t·km^{-2}·yr^{-1})	Area of production unit (km^2)	Annual production (t × 10^4)	Percentage ot total production	Total (t × 10^4)
Suspension feeders	0–10	1.19	204	0.02		
	10–100	27.70	428	1.19		
	100–1000	168.73	128	2.16		
	>1000	4092.71	20	8.19	67.64	11.56
Deposit feeders	<10	24.41	220	0.10		
	>10	33.01	560	1.85	11.41	1.95
Others	—	4.95	780	0.39	2.28	0.39
Mixed intertidal	—	100.00[a]	319	3.19	18.67	3.19
					100	= 17.09

[a]Estimated.

TABLE 4. Observed and predicted secondary benthic production of suspension-feeding macrofauna in the lower Bay of Fundy. The estimates are for 56 stations for which tidal current (y) and macrobenthic production (x) data are available and the fitted regression $\log_{10} x = -1.38 + 0.06y$ from Table 1. The area of the lower Bay excluding the intertidal zone is 8040 km^2.

Environmental conditions	Mean production per station + SE (t·km^{-2}·yr^{-1})	Total annual production (t × 10^4)
Without B9: predicted	180.23 ± 34.03	144.90
Without B9: observed	218.69 ± 63.33	175.83
With B9: predicted	148.90 ± 23.25	119.72

observed quantitative macrobenthic data. The observed production estimates for 1978–79 in Table 4 are higher than previously given by Wildish and Peer (1983) because of the selection of only 56 stations, compared with the original 98. As with most benthic biomass data, the mean values are associated with rather high variances as shown by the high standard errors of Table 4. Comparing predicted and observed production with and without the B9 barrage in place shows no significant difference between the means ($p < 0.05$). Macrobenthic suspension-feeding animal production is predicted to be 17% less with the B9 barrage in place than with it absent, but this difference is small enough to be within the sampling error associated with benthic sampling.

The change in curent speed resulting from the presence of the B9 barrage will also affect the interaction between currents and the sedimentary regime. This can be approximated from the changes expected in tidally averaged bed stress values throughout the Bay (Fig. 6). Again, the major changes would occur in Minas Basin and Cobequid Bay, with the area behind the barrage becoming a net sedimenting area, encouraging the development of deposit-feeding animals. Possible changes in the sedimentary regime resulting from the B9 barrage have been considered in some detail by Amos and Long (1980) and Amos

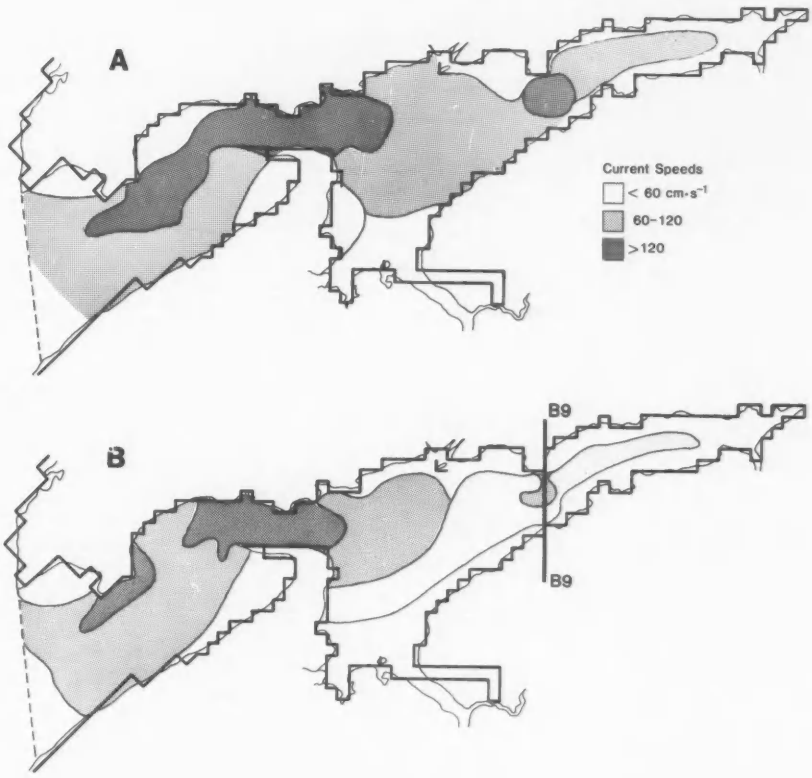

FIG. 5. Average tidal current speed in Minas Basin (A) without and (B) with the B9 barrage (data from Greenberg 1976, 1979).

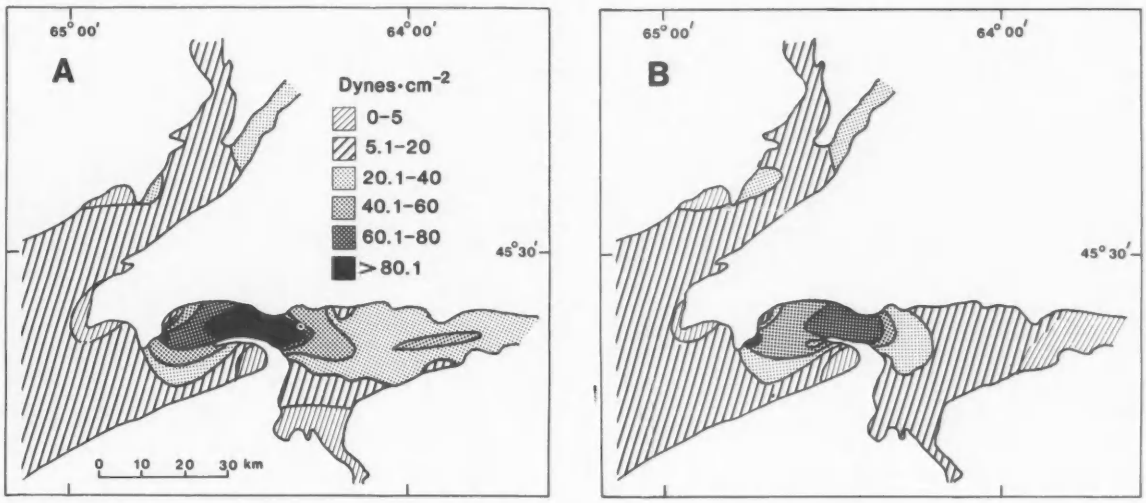

FIG. 6. Average bed shear stress, τ_0, for upper Bay of Fundy sediments (A) without and (B) with the B9 barrage in Minas Basin (based on data in Fig. 5).

TABLE 5. Total macrofaunal production in the Bay of Fundy (t carbon·yr^{-1} × 10^3, assuming 1 t wet wt ≈0.1 t carbon).

Geographic area	Trophic Group				
	Suspension-feeding	Deposit-feeding	Others	Mixed intertidal	Total
Lower Bay	147.23	10.55	4.30	4.62	166.70
Chignecto	11.56	1.95	0.39	3.19	17.09
Minas	34.74	0.36	1.10	5.38	41.58
Total	193.53	12.86	5.79	13.19	225.37
Percentage	85.9	5.7	2.6	5.8	100.0

and Asprey (1979) for the Minas Basin and Chignecto Bay, respectively.

Discussion

The production data presented here are based on individual estimates of the lifespan for each species and its inverse logarithmic relationship with $P:B$ as calculated by Robertson (1979). The lifespan estimates are based on literature sources and local knowledge, when available, so that varying levels of lifespan accuracy are included in the calculations. An alternative method, that of Banse and Mosher (1980), utilizes the relationships between size at maturity and $P:B$. Because size at maturity was not known for most species, this method could not be used for this study. However, a modified form of it, in which the mean biomass calculated from the total biomass divided by the density of each species at a station (w), was used in the equation $P:B = 0.112w^{-0.3}$ (Banse and Mosher 1980). Production calculated by this equation is approximately one half of that calculated by the Robertson equation, although total production at each station was related to the Robertson estimate (R) by the regression $-P:B = -6.57 + 0.43R$, $n = 135$, $r = 0.90$. Thus, the modified size estimate of production yielded lower absolute values but was equivalent proportionally to the Robertson estimate over three orders of magnitude. For amphipods, Wildish (1984) found that the Robertson method gave values of $P:B$ closer to the more accurate individual population cohort summation of losses and size frequency estimates than did the Banse and Mosher method. The latter was found to be oversensitive to both size differences and the conversion from wet weight to kilocalorie biomass. However, the modified Banse and Mosher method has the advantage that all of the necessary information for calculation of $P:B$ can be obtained directly from the data without resorting to the literature.

Prouse et al. (1984) have estimated the amounts of primary production, in units of tonnes carbon per year × 10^3, originating in the Bay of Fundy. In comparison, we present secondary, heterotrophic, macrofaunal production for the Bay converted from wet biomass production per year to these same units by multiplying by 0.1 (Table 5). Total macrofaunal production is 225 compared with a total primary production of 1125 t carbon·yr^{-1} × 10^3. Of the latter, nearly 96% was phytoplankton, 2.4% macroalgae, 1.24% microalgae, and the small remainder, marsh vegetation. Very little is known about the transfer of this primary production to tertiary levels of the food web, although a start has been made in examining pelagic–benthic coupling (Emerson et al. 1986) in the southwest mouth of the Bay of Fundy.

Clearly, our methods are not fully satisfactory for predicting the biological consequences of large-scale civil engineering works such as tidal barrage construction because quantitative predictions cannot be made for the most affected part of the Bay of Fundy: Minas Basin. Our observation that suspension-feeding animal production was a linear function of tidal current speed applied only in the lower Bay of Fundy, and even there not all stations fitted that model. The eliminated stations showed evidence of high sediment shear stress, suggesting that settlement and growth of suspension feeders was hindered by the sediment − water current interaction. In the upper Bay a lack of significant relationship between suspension-feeding animal production and current speed could be attributed to the extremes in many of the physical parameters. In Minas Basin, these would include sediment shear stress and the very high levels of suspended sediment present. Kiørboe et al. (1981) showed experimentally that high levels of suspended bottom material inhibit growth of the blue mussel, *Mytilus edulis*. This interpretation is in line with a multiple limiting factor concept because many physical or biological factors are thought to limit the production of macrofauna. Little attention has been paid to the population response, in terms of production, by suspension-feeding animals, to physical factors such as current speed and suspended sediment load. Such responses appear to be, typically, a reverse ramp function with an optimum and upper limiting level; for example, the population growth response by blue mussels to increased turbidity (Kiørboe et al. 1981) and the growth response by scallops, *Placopecten magellanicus*, to increasing current speed (Wildish et al., in prep.). Our field observations in Minas suggest that suspension-feeders, such as horse mussels, are inhibited by the highest tidal currents which occur in the main channel near Cape Split, although barnacles with an excellent means of attaching to the substrate can exist here. Further field and laboratory experiments are required to examine the importance of these physical factors on production, including an examination of their interactive effects.

Qualitative changes in Minas can be predicted, such as a loss of much of the production by horse mussels and other suspension-feeding macrofauna in Cobequid Bay, and their replacement by deposit-feeding infauna both here and landwards of the proposed B9 barrage. Because suspension-feeding animals are proportionately more important in the production budgets of the Bay of Fundy (Table 5), it is suggested that the B9 barrage would result in lower productivity of the upper Bay of Fundy.

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Analysis of Long-Term Ecological Data Using Categorical Time Series Regression

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We propose a time series analysis method based on the use of categorized variables and ordinary least squares regression. It has several advantages over Box–Jenkins models and time series regression with continuous variables, including model specification based on ecological information, parsimonious representations of the functional forms of model terms and interactions, robust treatment of the high uncertainty associated with long-term ecological data, and interpretive features based on linear combinations of the regression coefficients. Aspects of model building, significance testing, and interpretation of results are discussed and illustrated with a fisheries example involving an annual measure of white perch (*Morone americana*) stock size in the Delaware River/Bay from 1929 to 1974. Variation in white perch dynamics is analyzed using the following explanatory variables: lagged values of stock, hydrographic variables (freshwater flow and water temperature), and pollution-related variables (sewage loading, dredging activity, and dissolved oxygen). Potential statistical problems with the new method involving multicollinearity, autocorrelated errors, and other violations of ordinary least squares are identified.

Les auteurs proposent une méthode d'analyse par séries chronologiques basée sur l'utilisation de variables catégorisées et de la régression normale par moindres carrés. Cette méthode présente plusieurs avantages par rapport aux modèles de Box–Jenkins et à la régression par séries chronologiques à variables continues, notamment la spécification du modèle basée sur des données écologiques, la représentation parcimonieuse des formes fonctionnelles des termes et des interactions du modèle, le traitement vigoreux du haut niveau d'incertitude liée aux données écologiques à long terme et certaines caractéristiques d'interprétation basées sur des combinaisons linéaires des coefficients de régression. Les auteurs traitent de certains aspects de l'élaboration du modèle, du test de signification et de l'interprétation des résultats, et les illustrent par un exemple de pêche portant sur la mesure annuelle de la taille du stock de barets (*Morone americana*) dans le fleuve et la baie Delaware de 1929 à 1974. Ils analysent la variation de la dynamique du stock de barets à l'aide des variables suivantes : valeurs décalées du stock, variables hydrographiques (débit des eaux douces et température de l'eau) et variables liées à la pollution (déversement d'eaux usées, dragage et oxygène dissous). Les auteurs indiquent les problèmes d'ordre statistique que pourraient poser la nouvelle méthode et qui ont trait à la multicoliénarité, aux erreurs autocorrélées et à d'autres écarts par rapport à la méthode normale des moindres carrés.

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Ecological data often consist of time series of a response variable (or variables) and multiple explanatory variables (e.g. Beeton 1969; Jonasson 1972; Mahloch 1974; Goldman 1981; Edmondson and Litt 1982; Smith and Eppley 1982; Holland and Kjerfve 1985). Typically there is a great deal of uncertainty associated with these data, yet these data are often analyzed with the objective of explaining the variability observed in the response variable by the variability in the explanatory variables. Results from such analyses have been used to (1) identify variables that greatly influence the response variable (e.g. Botsford and Wickman 1975; Holland 1985), (2) predict unobserved values of the response variable (e.g. Austin and Ingham 1976; Stocker and Hilborn 1981), or (3) detect changes in the response variable time series (e.g. Hipel et al. 1975; Millard et al. 1985).

In some situations, the response variable, appropriately lagged back in time, also appears as an explanatory variable. Two commonly used approaches for analyzing time series data with lagged response variables as explanatory variables are Box–Jenkins models and time series linear regression.

The approach underlying Box–Jenkins models (Box and Jenkins 1976) is to remove empirically as much structure from the data as possible, with the ultimate goal of being left with only "white noise" in the residuals. The general procedure involves first transforming the data to obtain a stationary time series and then analyzing the transformed data to obtain uncorrelated errors. Box–Jenkins models can yield excellent empirical representations of the behavior of the response variable time series. While empirical representation of the response variable time series is desirable for forecasting, it is not always appropriate for analyzing ecological data with the objective of partitioning variability (especially trend) of the response variable to individual explanatory variables based on postulated ecological principles. Although in theory, Box–Jenkins models can be specified a priori, rather than on information empirically derived solely from the data, this is typically not

done in practice.

Time series regression has been used to analyze trends in nonstationary time series data (Johnston 1972; Ostrom 1978), and thus may be better suited for statistical model building based on ecological information than Box–Jenkins methods. Ecologically based statistical models can be postulated and regression analysis of the data used to test their utility. However, there are drawbacks associated with time series regression. One or more of the assumptions of least squares estimation can be violated by the inclusion of the response variable as an explanatory variable or by the presence of autocorrelated errors. Such violations of assumptions can result in biased and/or inconsistent estimates of the regression coefficients. Also, the regression diagnostics used to detect such problems as autocorrelation among the residuals (e.g. Durbin–Watson statistic) can produce misleading results.

Perhaps of greater consequence to ecologically based analyses are the difficulties associated with model building using time series regression. The number of terms in the model can quickly become cumbersome when multiple time lags of several explanatory variables are included. Model building using the standard regression approaches would result in a term for each lag of each explanatory variable, and estimation of any interaction effects among the explanatory variables would require a specific term for each interaction. Another problem is that the functional form of each term in the model (e.g. linear, exponential) must be specified. When models contain a large number of terms, there may be no feasible method for statistically selecting the proper functional form of each term. Clearly, useable time series regression models would be those with only a few explanatory variables, a limited number of time lags, and only a few interaction terms.

In this paper, we present a time series analysis method based on ordinary least squares (OLS) regression and categorized variables. The method is conceptually similar to that proposed in a nontime series context by Breiman et al. (1984). The proposed method enables the partitioning of response variability (including trend) among individual explanatory variables, accommodates model building based on ecological information, and allows for parsimonious representation of direct (main) effects and interaction effects among the explanatory variables. In addition, the method provides for robust treatment of the high uncertainty usually associated with ecological data. We first present the proposed time series regression model in general terms. Next, we describe model significance testing, interpretation, and model building (including a procedure for selection among explanatory variables). We then present an example using historical fisheries landings and hydrographic and pollution variables to illustrate the application of the model. Finally, the utility of the method is discussed using the fisheries example, and the major unresolved issues involving statistical aspects of the method are outlined.

Proposed Categorical Time Series Regression Model

Model Structure

The first step in the analysis is to categorize the observations of the response variable and each of the explanatory variables time series based on some criterion. For illustrative purposes, we use two categories (denoted 1 and 2), based on the median value of each time series. Use of the median is arbitrary, and provided there are sufficient data, any number of categories can be defined based on a variety of possible criterion. Breiman et al. (1984) provide an excellent discussion on alternative criteria for categorizing the explanatory variables, which, in their nomenclature, are termed "splitting rules."

Using t to denote time, assume we have the original response variable (Y_t), the categorized response variable time series (Z_t), and $n-1$ categorized explanatory variable time series (X_{it}, $i = 1, n-1$). We define a new variable (V_i), corresponding to each X_{it}, such that V_i equals the values assigned to the categories of X_{it} (i.e. for two categories, if $X_{it} = 1$ or 2, then $V_i = 1$ or 2). Treating the categorized response variable as the nth explanatory variable (i.e. X_{it}, $i = 1, n$ where $X_{nt} = Z_t$), the regression model is of the form

$$(1)\quad Y_t = \sum_{m=q}^{p} \sum_{V_1=1}^{2} \sum_{V_2=1}^{2} \cdots \sum_{V_n=1}^{2} \times (B_{V_1,V_2,\ldots,V_n} \cdot W_{t-m,V_1,V_2,\ldots,V_n} + e_{V_1,V_2,\ldots,V_n})$$

where

$$W_{t-m,V_1,V_2,\ldots,V_n} = \begin{cases} 1/(p-q+1) \text{ if } X_{1,t-m} = V_1, \\ \qquad \text{and } X_{2,t-m} = V_2, \\ \qquad \vdots \\ \qquad \text{and } X_{n,t-m} = V_n \\ 0 \qquad \text{otherwise} \end{cases}$$

and $B_{V_1,V_2,\ldots,V_n}$ = regression coefficients, $e_{V_1,V_2,\ldots,V_n}$ = errors, p = earliest (or furthest back in time) lag in the model, and q = latest (or most recent) lag in the model.

In the case of two categories, the method involves defining 2^n cells, which cover all possible combinations of the categorized explanatory variables (i.e. values of each variable can either be high or low). Using this method, we look back in time, within the window of time defined by the earliest and latest lags, at the levels of the categorized explanatory variables. The number of times that the conditions defined by each cell occur in the lagged observations corresponding to a given time t (i.e. between times $t-p$ and $t-q$) is calculated. These are then divided by the number of lags in the model ($p-q+1$). For a total of T observations (i.e. $t = 1, 2, \ldots, T$), the result is a $T \times 2^n$ design matrix in which each row represents a value of time and each column is associated with a cell. Each element of the matrix is the number of occurrences of those categories of explanatory variables defined by the cell, divided by the number of lags, which were observed in the lagged data associated with time t. The original response variable time series is then regressed on this design matrix using OLS estimation.

Model Testing and Interpretation

The significance of a particular model can be tested with an F-statistic (regression mean square/residual mean square) of the null hypothesis H_0: all $B_{V_1,V_2,\ldots,V_n} = 0$. However, since the proposed regression model has no intercept term (see eq. (1)), the usual coefficient of determination (R^2) measuring the fit of model predictions to observed data calculated from regression sums-of-squares information is artificially inflated (Arnold and Good 1981; Kvalseth 1985). An appropriate R^2 can be obtained by reapplying the same model to the data but with an intercept term. The regression coefficients obtained from the model with an intercept are then deviations from the overall mean (rather

than the means themselves in the nonintercept model), and linear combinations of them correspond to the coefficients from the nonintercept model.

An important feature of the regression model is its parsimonious treatment of interaction effects among the variables. By using categorized variables, a regression coefficient ($B_{V_1,V_2,\ldots,V_n}$) is associated with each possible combination of variables similar to the ANOVA of a factorial design. A regression coefficient corresponding to the main effects in ANOVA can be obtained by collapsing over appropriate cells (i.e. adding together appropriate $B_{V_1,V_2,\ldots,V_n}$). A test comparing any two regression coefficients, or any linear combination of coefficients, can be performed based on confidence intervals calculated using variance estimates from the variance/covariance matrix of the $B_{V_1,V_2,\ldots,V_n}$. In this manner, the direct effects of each explanatory variable on the response variable (i.e. averaged over all variables in the model) can be calculated and compared with any combination of interaction effects. Thus, categorical regression provides a parsimonious representation of interaction effects without requiring specification of the functional forms in the model. Also, categorical regression allows investigations and comparisons of main effects and interactions via linear contrasts of the regression coefficients.

Another interpretive feature of the categorical regression time series model is that time lags are all treated identically. In eq. (1) there is a $B_{V_1,V_2,\ldots,V_n}$ for each of the 2^n cells (columns in the design matrix). Coefficients are not specific to any particular lag; rather, the model averages over time lags. Thus, each $B_{V_1,V_2,\ldots,V_n}$ is interpreted as the average contribution to the present value of Y_t from the conditions associated with that cell (i.e. defined by the values of $V_1, V_2, \ldots, V_n$), given the observed history of the explanatory variables. This is the reason for dividing by the number of lags ($p - q + 1$) in eq. (1). With coefficients estimated in this manner, the combinations of explanatory variables which lead to greater and less than average contributions to the present values of the response variable can be identified and statistically compared with the overall average contribution (i.e. average of all $B_{V_1,V_2,\ldots,V_n}$) using a t-test.

Finally, in addition to the usual sources of variability observed in ecological data (e.g. limited sampling frequency, patchiness), the construction of long-term data sets usually requires synthesis from a variety of sources and can involve data obtained using different measurement methods. Use of categorized variables addresses the great uncertainty typically found in long-term ecological data. We are more apt to believe historical data characterized by categories (e.g. high and low) than by the specific values of the variables.

Model Building

Determination of the "best" model requires a systematic method of model building, especially when numerous candidate explanatory variables are available. We recommend that the first step in model building be the a priori specification of a candidate set of explanatory variables and reasonable lags based on known or hypothesized ecological information. The approach to model building would depend on the number of candidate variables specified. If there were only a limited number of candidate variables, then some (or all) combinations of variables could be tested. If a substantial number of variables are available as candidate explanatory variables, then a variable selection procedure is needed. We have utilized a procedure similar to the forward selection method used in linear regression (Kleinbaum and Klupper 1978). First, the model is applied to each candidate explanatory variable (i.e. for two categories, $2^1 = 2$ cells) and the residuals are checked for autocorrelation using a runs test (Draper and Smith 1981). We use a runs test, rather than the commonly used Durbin–Watson statistic, because in the situation of a lagged response variable appearing as an explanatory variable and autocorrelated errors, the Durbin–Watson statistic is an insensitive indicator of autocorrelation (Ostrom 1978). Based on the F-test for model significance, possible models are identified. Using the R^2 values and the results of the runs test, the "best" model is selected. Second, using the "best" model, each of the remaining candidate variables is added singly, and the model which shows a significant increase in the amount of variation explained is then designated as the "best" model. The increase in the amount of variation explained can be examined using an F-test (see Neter and Wasserman 1974, general linear test) based on residual sum of squares (RSS) from the full (f) and reduced (r) models:

$$(2)\quad F_{[\mathrm{df(r)}\ -\ \mathrm{df(f)}],\,\mathrm{df(f)}} = \frac{\mathrm{RSS(r)} - \mathrm{RSS(f)}}{\mathrm{df(r)} - \mathrm{df(f)}} \cdot \frac{\mathrm{df(f)}}{\mathrm{RSS(f)}}.$$

This process continues until none of the remaining candidate variables can significantly increase the amount of variation explained over the previous model. This procedure is by no means rigid, and ecological information can be used at any step to eliminate some models from consideration and/or preferentially test other models. Furthermore, alternative criteria than R^2 values can also be used as the basis for model building (e.g. see "optimal pruning" in Breiman et al. 1984).

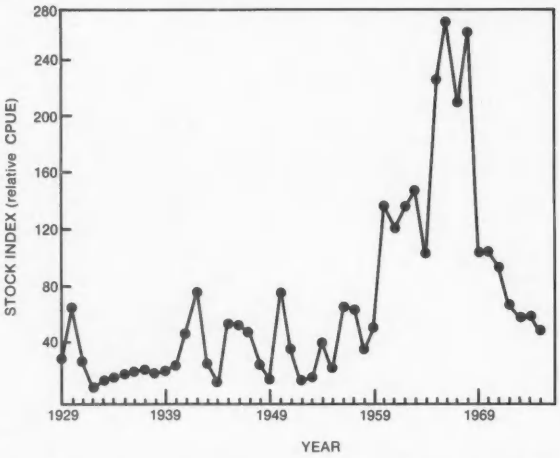

FIG. 1. Annual white perch stock index in the Delaware River/Bay constructed from commercial landings and effort data.

A Fisheries Example

The example presented here involves white perch (*Morone americana*) landings from the Delaware River/Bay and serves to illustrate the utility of the categorical time series regression method in the analysis of ecological data. Additional applications of the proposed time series method to fisheries data can be found in Summers et al. (1987).

The objective of the analysis is to examine the influences of hydrographic and "pollution" variables on white perch stock dynamics. The response variable is a catch-per-unit-effort-like

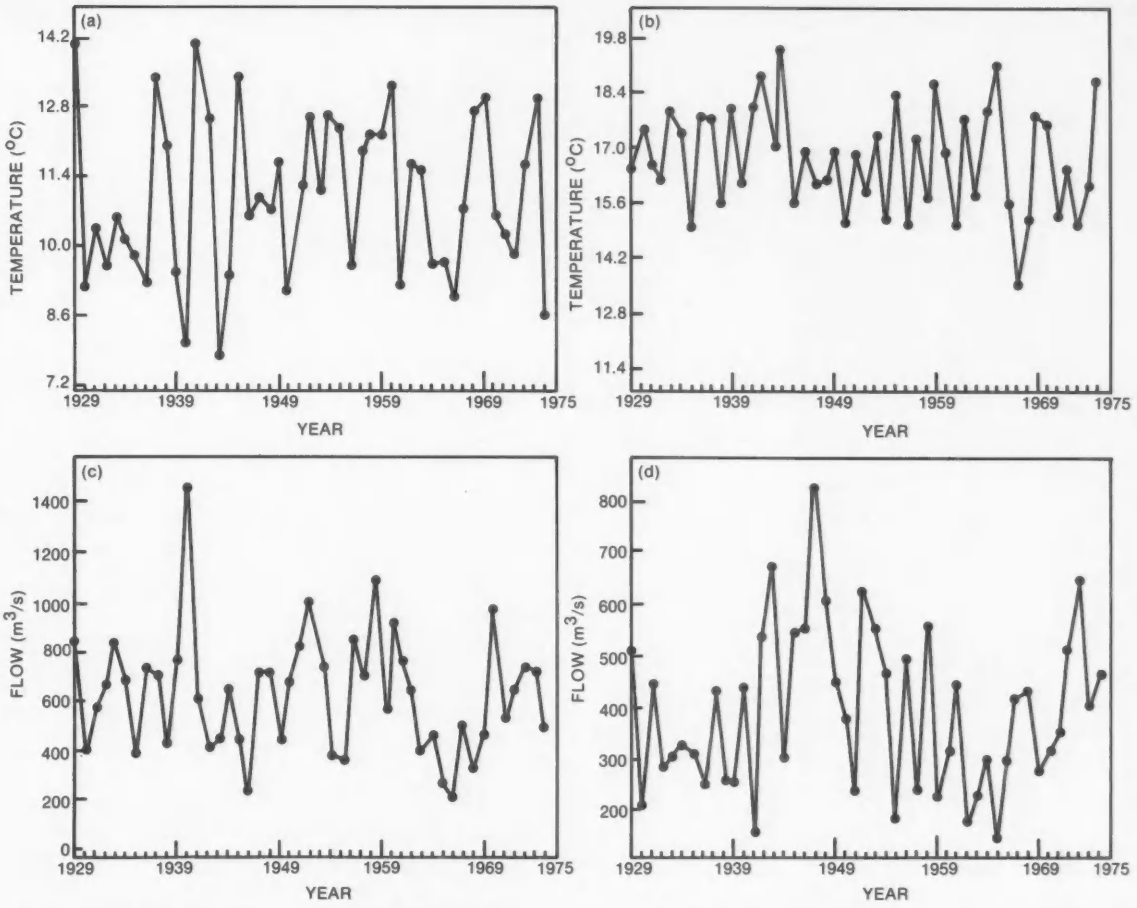

FIG. 2. Hydrographic explanatory variables in the time series regression model. Average monthly water temperature and freshwater flow in the Delaware River/Bay at Trenton, NJ. (a) April temperature; (b) May temperature; (c) April flow; (d) May flow.

index of white perch fish stock in the Delaware River/Bay obtained from annual commercial landings and effort for 1929–74 (see Summers et al. (1985) for derivation of the stock index). The available hydrographic variables are monthly water temperature and flow in the Delaware River at Trenton, NJ. The pollution variables are the annual sewage loading to the Delaware from treatment plants in Philadelphia, PA, and Camden, NJ, minimum average summertime dissolved oxygen concentration between river km 77 and 216, and the annual volume of material dredged between river km 0 and 217. The sewage loading and dissolved oxygen variables are only very gross indicators of anthropogenic related pollution trends in the watershed. The fishery consists of fish ages 3–8, and spawning takes place during April and May (Public Service Electric and Gas Co. 1984; Hardy 1978). We postulated that the value of the stock index in any given year should be the result of spawning for all prior years which contributed to the stock index in that year. Furthermore, the magnitude of success of spawning in a given year should depend on the size of the spawning stock and the hydrographic and pollution conditions on the spawning grounds during the period of spawning and early development. This latter assumption is based on the premise that very early lifestages are the most sensitive to environmental conditions; thus, hydrographic and pollution conditions during, and just after, spawning would be expected to have the greatest influence on year class strength. Based on this information, we identified the candidate explanatory variables as stock index, average April freshwater flow and temperature, average May freshwater flow and temperature, and the three pollution-related variables, all lagged 3–8 yr. The response and explanatory variable time series are shown in Fig. 1, 2, and 3.

We implemented a model building scheme which would result in conservative conclusions concerning the roles of hydrographic and pollution variables in controlling white perch stock dynamics. The model building scheme first removes the variability explained by lagged stock index, then adds hydrographic variables, and finally, adds pollution indicator variables. By sequentially adding variables in this manner, we ensure that any variability explained by pollution variables could not be explained alternatively by stock or hydrographic variables. Figure 4 shows the results from the model building procedure. The first model is with lagged stock only. Next, with lagged stock in the model, each of the candidate hydro-

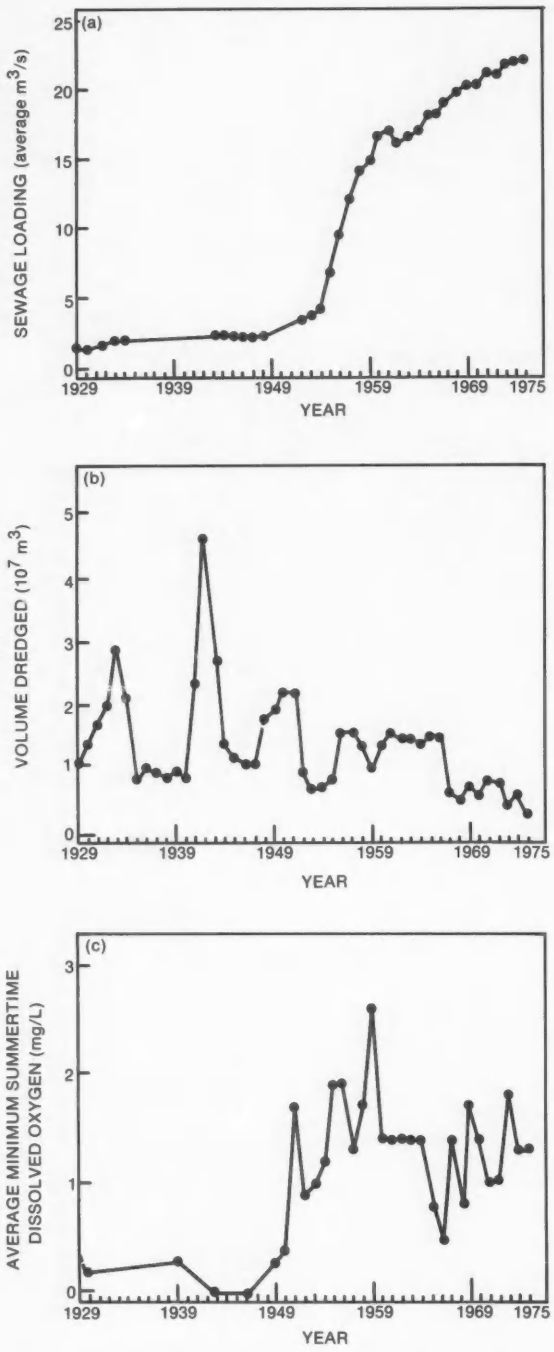

FIG. 3. Pollution trend explanatory variables in the time series regression model. (a) Annual sewage loading to the Delaware River from sewage treatment plants in Philadelphia, PA, and Camden, NJ; (b) annual volume of material dredged between river km 0 and 217; (c) minimum average summertime dissolved oxygen concentrations between river km 77 and 216.

TABLE 1. Estimated values and 95% confidence intervals (CI) for the eight B_{V_1,V_2,V_3} from the "best" white perch categorical time series model. The subscripts are defined as V_1 denoting May flow, V_2 denoting April temperature, and V_3 denoting lagged stock. The values of V_i are defined as a value of 1 indicating the low category and a value of 2 indicating the high category. Included is the 95% CI for the overall average of the coefficients. *Significantly different from overall mean: **Significantly different from all coefficients and from the overall mean, $p < 0.05$.

Coefficient	Estimated value	95% CI
B^{*}_{111}	18.4	(−20, 57)
B_{112}	43.1	(−19, 105)
B_{121}	26.7	(−72, 126)
B^{**}_{122}	369.2	(315, 423)
B_{211}	18.2	(−97, 133)
B^{*}_{212}	−141.5	(−286, 3)
B_{221}	132.9	(54, 212)
B_{222}	109.8	(−15, 235)
Overall mean	72.1	(66, 78)

graphic variables was added. The F-statistic for testing the significance of the reduction in the RSS due to the addition of the each hydrographic variable was calculated. The model with lagged April temperature and lagged stock was then selected, since it resulted in a significant decrease in the RSS and produced the highest R^2. Each of the remaining explanatory variables was then added to the model of lagged April temperature and lagged stock and the F-statistic and R^2 values calculated. The model with lagged May flow, April temperature, and stock was selected, since May flow was the only hydrographic variable which significantly reduced the RSS. Furthermore, the runs test for autocorrelation among the residuals was not significant. Finally, each of the pollution-related variables was added to the model with lagged May flow, April temperature, and stock. None of the pollution variables could explain a significant portion of the remaining 18% of the variability in the stock series. Thus, we conclude that the "best" model is lagged May flow, April temperature, and stock. Model predictions from the "best" model are compared with the observed white perch stock series in Fig. 5.

Having decided on the "best" model, we proceed with the interpretation of the coefficients. Table 1 shows the values of the eight B_{V_1,V_2,V_3} with their 95% confidence intervals. The subscripts are defined as V_1 denoting May flow, V_2 denoting April temperature, and V_3 denoting stock. The values of V_i are defined as a value of 1 indicating the low category and a value of 2 indicating the high category. Included in Table 1 are the 95% confidence intervals for the grand average of the coefficients. The conditions of low May flow, high April temperature, and high stock (B_{122}) contributed to future values of stock significantly greater than any other combination of conditions and also greater than the overall average. The conditions of low May flow, low April temperature, and low stock (B_{111}) and high May flow, low April temperature, and high stock (B_{212}) contributed to future values of stock significantly less than the overall average.

Table 2 shows the main effects and their 95% confidence intervals associated with each variable. The individual values of B_{V_1,V_2,V_3} summed to obtain each main effect are also shown. Using the effect of low May flow as an example, the standard errors of the main effects used for generating confidence intervals are calculated as:

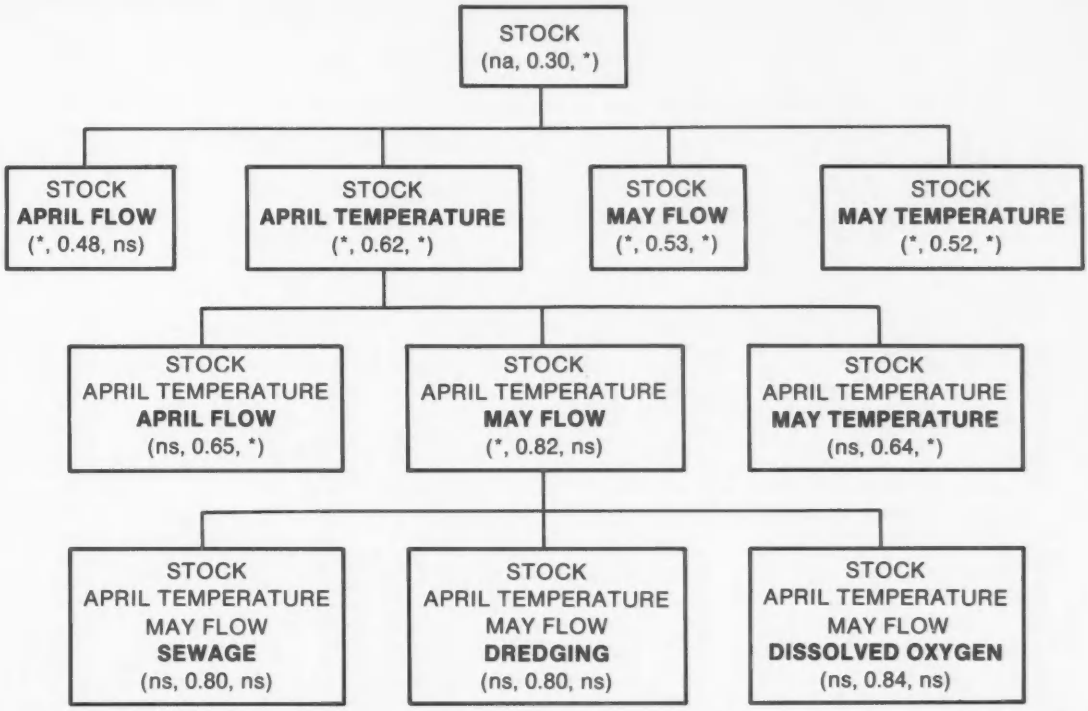

FIG. 4. Results of the model building scheme implemented for the categorical time series regression analysis of white perch stock dynamics. The variables shown in bold are the new variables, lagged 3–8 yr, added to the model at that step. The three values in parentheses are the significance of the F-test for reduction in RSS, the coefficient of determination between model predictions and observed values adjusted for degrees of freedom (R^2), and the significance of the runs test for autocorrelated residuals (*$p < 0.05$; ns = not significant; na = not applicable).

$$(3)\quad \mathrm{SE}(\mathrm{EFFECT}_{\text{low May flow}}) = \{\mathrm{var}(B_{111}) + \mathrm{var}(B_{121}) + \mathrm{var}(B_{112}) + \mathrm{var}(B_{122}) + 2[\mathrm{cov}(B_{111}, B_{121}) + \mathrm{cov}(B_{111}, B_{112}) + \mathrm{cov}(B_{111}, B_{122}) + \mathrm{cov}(B_{121}, B_{112}) + \mathrm{cov}(B_{121}, B_{122}) + \mathrm{cov}(B_{112}, B_{122})]\}^{1/2}.$$

Table 2 shows that the effects of high April temperature and low May flow in producing high future values of stock are significant after averaging over the remaining variables. Stock does not show a significant main effect, but under the conditions of low May flow and high April temperature, high stock conditions produce a significantly greater contribution to future stock than do low stock conditions (see Table 1, B_{122} compared with B_{121}).

The "best" categorical time series regression model (lagged May flow, April temperature, and stock) can also be interpreted in the context of the biology of white perch, although cause and effect relationships are obviously difficult to conclude from statistical (correlative) results. Interpretation is further complicated because the analyses are based on an index of stock size which consists of multiple age classes rather than on year (or age) class specific data.

Analyses showed significant statistical relationships between hydrographic variables during the spawning period (May flow and April temperature) and future values of white perch stock. Whether these relationships are due to direct effects of flow and temperature on egg hatching success and early lifestage survival, or alternatively, flow and temperature are surrogates for other variables or environmental conditions which affect white perch, is unknown. Water temperature has been shown to directly affect hatching success in white perch with an optimal temperature of approximately 14°C (Morgan and Rasin 1982).

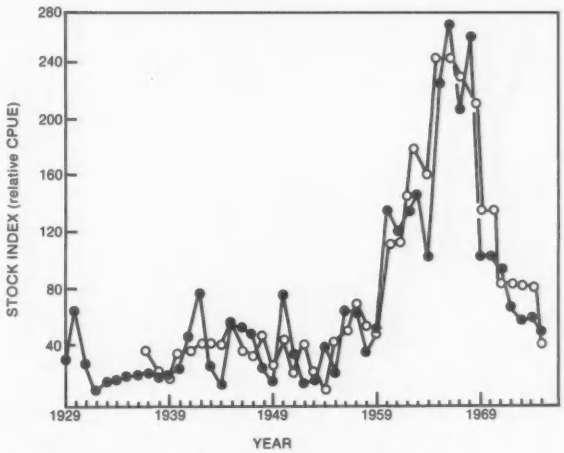

FIG. 5. Comparison of observed (●) and predicted (○) values of the white perch stock index. Predicted values are from the "best" categorical time series model (lagged stock, April temperature, and May flow).

TABLE 2. Main effects and their 95% confidence intervals (CI) for each variable in the "best" time series categorical regression model of white perch (lagged May flow, April temperature, and stock). The significance of main effects is shown for each variable (*$p < 0.05$; ns = not significant).

Variable	Level	Combination of coefficients	Main effect	95% CI
Stock (ns)	Low	$B_{111} + B_{121} + B_{211} + B_{221}$	196.2	(94, 298)
	High	$B_{112} + B_{122} + B_{212} + B_{222}$	380.6	(272, 488)
April temperature*	Low	$B_{111} + B_{112} + B_{211} + B_{212}$	61.8	(−141, 263)
	High	$B_{121} + B_{122} + B_{221} + B_{222}$	638.6	(456, 820)
May flow*	Low	$B_{111} + B_{112} + B_{121} + B_{122}$	457.0	(345, 569)
	High	$B_{211} + B_{212} + B_{221} + B_{222}$	119.4	(9, 229)

In our analysis, April temperature is categorized into low and high values based on the median (low 7.8−10.9°C and high 10.9−14.2°C). High April temperature, which is closer to the optimal temperature for hatching success than low April temperature, is associated with high future values of stock. Water temperature is known to also directly affect larval fish development rates (Lasker 1981) and has been suggested as affecting the availability of white perch prey (Mansueti 1961), both of which can affect larval survival.

Several alternative interpretations of the statistical relationship between May flow and future values of stock are also possible. White perch spawn in the tidal fresh to slightly brackish tributaries and embayments of estuaries (Hardy 1978). Eggs typically sink to the bottom and become attached to grass, rock, and other debris, although some eggs float free (Hardy 1978). River flow can directly affect the amount and quality of habitat physically available for spawning and egg settlement as well as the number of eggs which remain demersal. Location of egg deposition can, in turn, affect the amount and type of white perch prey and predators. River flow is also likely related to salinity and suspended sediment concentrations, which may directly affect hatching success, early lifestage development, and the availability of white perch prey (Mansueti 1961; Auld and Schubel 1978; Morgan and Rasin 1982; Morgan et al. 1983).

Discussion

Utility of the Proposed Method

The white perch data presented above demonstrate the problems facing ecologists attempting to decipher trends and identify important controlling variables when data consist of multivariate time series. The high uncertainty and multiple lag structure commonly found in fisheries data, and in other ecological situations, prevent intuitive interpretation. This is seen by examination of the response variable and explanatory variable time series for the white perch example (Fig. 1−3). Visual inspection of the hydrographic variable time series (April flow and temperature, May flow and temperature) does not indicate any obvious relationships to the stock series. Yet, using categorical time series regression, 62% of the variability in the white perch time series can be explained with the combination of previous stock conditions and April temperature; 82% can be explained with previous stock, April temperature, and May flow. Furthermore, the inclusion of additional explanatory variables (pollution-related variables) does not significantly increase the amount of variation explained. Inspection of the sewage loading and dissolved oxygen pollution time series variables (Fig. 3a and 3c) shows that they could be construed to be likely candidates for explaining the "apparent" upward trend of the white perch stock series. Categorical time series regression analysis showed that using the lagged stock series and the seemingly trendless hydrographic variables, the upward trend of white perch can be explained without the inclusion of sewage loading or dissolved oxygen. Also, the explanation of the trend is simply not due to having lagged stock in the model, since using only lagged stock we obtain a $R^2 = 0.30$. It is with the addition of the two hydrographic variables that R^2 increases to 0.82.

The analysis also showed that, in general, low May flow and high April temperature during the spawning period resulted in relatively high future values of stock. The analysis identified specific sets of conditions which produce high future values of stock (low May flow, high April temperature, high stock) and low future values of stock (low May flow, low April temperature, low stock and high May flow, low April temperature, high stock). The apparent inconsistency in low May flow generally producing high future values of stock, and yet low May flow with low stock and low April temperature producing significantly less than average contributions to future values of stock, shows the importance of estimating interactions among the explanatory variables in explaining white perch stock dynamics. This type of situation can arise because estimation of the main effects involves an averaging of regression coefficients. Examination of specific conditions in the white perch example shows that low May flow, low April temperature, and low stock result in significantly low future values of stock when compared with other specific sets of conditions (see B_{111} in Table 1). Yet, for the main effects of low May flow, the magnitudes of the other coefficients more than compensate for the low value associated with the low May flow, low April temperature, and low stock coefficient, resulting in a high value for the main effect of low May flow.

Unresolved Statistical Issues

Several problems can arise with OLS estimation of time series regression models. These problems include collinearity among the columns of the design matrix, autocorrelated errors, and violations of least squares assumptions due to the lagged response variable appearing as an explanatory variable. Several situations involving various combinations of these problems are briefly reviewed below for time series regression using continuous data. The relevance of these potential problems of OLS estimation to the proposed categorical time series regression model is then discussed.

Collinearity among the columns of the design matrix creates

problems with the interpretation of the effects of individual explanatory variables on the response variable (Chatterjee and Price 1977). This problem is common to all regression analyses in which two or more of the explanatory variables are correlated. In standard regression models, each column of the design matrix corresponds to an explanatory variable so that correlations among explanatory variables translate directly into collinearity among columns of the design matrix. Collinearity can be of significant importance in time series regression models using continuous variables if multiple lags of the same variable are present as explanatory variables because this can result in highly correlated explanatory variables. For example, if an explanatory variable displays a monotonic trend, then lagged versions of the variable will be highly correlated with each other and with the original variable.

In the proposed time series model, collinearity among the columns of the design matrix can arise from two sources. First, collinearity can result from model misspecification causing two or more of the columns of the design matrix to contain a large number of zeroes. Model misspecification can be caused by either the number of explanatory variables or the combination of explanatory variables included in the analysis. The greater the number of explanatory variables included in the categorical model, the greater the number of cells (and hence elements) in the design matrix. For a given data set, this means that more of the elements of the matrix will be empty, increasing the possibility of collinearity among columns, since they could conceivably consist of many zero elements. Similarly, certain combinations of categorized explanatory variables may not have been observed in the data. This can also cause two or more columns in the design matrix to contain a large number of zeroes.

A second source of collinearity is correlation among two or more of the explanatory variables. This can be of particular importance, since multiple lags of the same time series are typically included in the analysis. In addition, by categorizing the data, we have reduced the amount of information (variability) contained in each explanatory variable. Explanatory variable time series for which differences as continuous variables were attributable to short-term, small-scale variation can become very similar when categorized. However, it is not clear how this reduction in information due to categorization affects the columns of the design matrix in the proposed model. When continuous variables are used, each column of the design matrix correponds to an explanatory variable. However, with the proposed model, the columns of the design matrix correpond to the frequency of occurrence of combinations of the categorized explanatory variables. The effect of categorization of the explanatory variables on the collinearity among columns of the design matrix is presently unclear and deserves further attention.

In time series regression with continuous variables, three situations involving autocorrelated errors and a lagged response variable appearing as an explanatory variable can be delineated. These are briefly summarized below from Ostrom (1978) and Johnston (1972) and the interested reader is referred to them for more details. The three situations are (1) autocorrelated errors without the lagged response variable as an explanatory variable, (2) uncorrelated errors and the lagged response variable included as an explanatory variable, and (3) autocorrelated errors and the lagged response variable included as an explanatory variable. Provided certain conditions on the autocorrelated errors hold (see Johnston 1972), use of OLS does not present major problems in situations (1) and (2). In these two situations, OLS results in consistent estimators, although they are biased in finite samples. The major problems arise when OLS is applied to situation (3). In this situation, OLS results in inconsistent and asymptotically biased estimates.

Our approach to the estimation of the proposed regression model is to use OLS and attempt to find a model without autocorrelated residuals (situation (2) above). This is desirable, since results with continuous variables have shown that, under certain conditions, this situation produces consistent and asymptotically efficient estimators (although the estimates are biased in finite samples). However, it is not clear how categorization affects results based on continuous variables. In time series regression using continuous data, the response variable is truly regressed on a lagged version of itself. In the proposed model, the continuous response variable is not directly regressed on itself but rather on the design matrix which includes frequencies of occurrences of conditions defined by the categorized response variable.

The consequences of categorization on collinearity among columns of the design matrix and on the properties of the estimators under various situations of autocorrelated errors and lagged response variables as explanatory variables need to be assessed. However, while there may be statistical problems with OLS estimation of the categorical regression model, these must be viewed in the context of the high uncertainty associated with the data and whether the data would satisfy assumptions underlying other statistical models.

Conclusions

We have presented a time series analysis method based on categorized variables and linear regression as an alternative to the more traditional approaches of Box–Jenkins models and regression using continuous variables. In general terms, and using the white perch example, we have attempted to demonstrate some of the features of the method including model specification based on ecological information, parsimonious representations of the functional forms of model terms and interactions, robust treatment of the high uncertainty associated with historical data, and interpretive aspects based on linear combinations of the regression coefficients. We also outlined some topics for future research involving the statistical properties of the method. We believe that categorical time series regression is a valuable method for analyzing variation in long-term ecological data. The method should not be viewed as replacing any particular existing methodology, but rather as another tool for the ecologists who must take "hard looks" at "soft" multivariate time series.

Acknowledgments

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Nickel Ion Requirements for Autotrophic Growth of Several Marine Microalgae with Urea Serving as Nitrogen Source

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Twelve marine species were tested for their competence to grow on urea as sole nitrogen source, and the influence of nickel ion on such urea utilization was determined. In those cases (six species) where good algal growth occurred in the seawater medium used without added nickel, the metal ion requirement was demonstrated by suppressing the algal growth with a chelator (citric acid) and testing for subsequent growth from addition of excess nickel. The results showed that a nickel requirement for urea utilization is of widespread occurrence among the microalgae tested. The concentrations of nickel tested in the laboratory were compared with the endogenous nickel levels reported for ocean waters, and it was concluded that these levels are generally sufficient to support an effective utilization of urea by most urease-producing algae. The value of chelation tests as a rapid, reliable method for the demonstration of the Ni^{2+} requirement is discussed.

Douze espèces d'algues microscopiques marines ont fait l'objet d'essais visant à déterminer leur capacité à croître en utilisant l'urée comme unique source d'azote, et l'effet de l'ion nickel sur cette utilisation a été déterminée. Dans les cas (six espèces) où une bonne croissance était obtenue en milieu à l'eau de mer sans addition de nickel, le besoin d'ions métalliques a été démontré par la suppression de la croissance à l'aide d'un chélateur (acide citrique) et par une reprise de la croissance par l'addition de nickel en excès. Les résultats obtenus montrent que le besoin en nickel pour l'utilisation de l'urée était généralisé chez les algues microscopiques étudiées. Les concentrations de nickel ayant fait l'objet d'essais en laboratoire ont été comparées aux concentrations de nickel endogène des océans et on a conclu que ces teneurs étaient généralement suffisantes pour permettre une utilisation efficace de l'urée par la plupart des algues produisant de l'uréase. Les auteurs traitent de la valeur des tests de chélation comme méthode rapide et fiable permettant de démontrer les besoins en Ni^{2+}.

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Urea is an excellent nitrogen source for photoautotrophic growth of marine microalgae both in culture and the natural environment (see Oliveira and Antia 1984 for review). Some of the anomalous cases of algae reported as unable to grow on urea or to utilize it poorly (e.g. McCarthy 1971; Antia et al. 1975) were later shown to grow well on this nitrogen source by increasing its concentration (Antia et al. 1977), or by optimizing the factorial combinations of light, temperature, and salinity parameters (Terlizzi and Karlander 1980), or by supplementing the medium with trace amounts of nickel ions (Oliveira and Antia 1984).

The available evidence shows that urea utilization by algae involves the production of either urease or ATP:urea amidolyase. Urease occurs exclusively in all algal classes excepting the Chlorophyceae where both enzymes are found but ATP:urea amidolyase is the more prominent (see Oliveira and Antia 1986 for review).

Of particular interest, in connection with urea utilization via urease, is the discovery that nickel is a constituent of jackbean (*Canavalia ensiformis* L.) urease (Dixon et al. 1975, 1976) and the subsequent finding that this metal ion is indispensable for the conversion of urea to ammonia in soybean tissue cultures (Polacco 1977). Synthesis and activity of urease in certain microalgae also depends on nickel (Syrett 1981; Rees and Bekheet 1982; Oliveira and Antia 1986). These observations suggested that the nickel requirement for microalgae to grow on urea as sole nitrogen source may be widespread, perhaps universal, among the members of all the urease-producing algal groups. Since information on this subject is lacking, we have reexamined the growth potential in nickel-enriched seawater media of some known urea utilizers such as those listed by McCarthy (1971) and Antia et al. (1975, 1977). To further our knowledge on the importance of Ni to urea utilization by marine phytoplankters, we have also included some species previously reported as unable or poorly able to utilize urea as a nitrogen source for growth (Droop 1955; Conover 1975).

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TABLE 1. Microalgae used in the present study.

Alga	Strain/clone (isolator/supplier/source)	Previous observations of growth on urea as nitrogen source
Prymnesiophyceae		
Hymenomonas elongata (Droop) Parke & Green	Droop, Millport No. 62	Growth doubtful[a]
Pavlova lutheri (Droop) Green	Droop, Millport No. 60	Good growth[b]
Prymnesium parvum Carter	Droop, Millport No. 65	No growth[a]
Bacillariophyceae		
Achnantes brevipes (Agardh) Cleve	Watson, clone No. 7	Not tested
Chaetoceros gracilis Schutt	Thomas, clone TO-58-2	Not tested
Skeletonema costatum (Greville) Cleve	Guillard, clone "Skel"	Good growth[c]
Thalassiosira nordenskioldii Cleve	Subba Rao, Nova Scotia Shelf	Poor Growth[d]
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	Guillard, clone "Actin"	Good growth[e]
Cryptophyceae		
Rhodomonas sp.	Lucas, clone unknown	Not tested
Dinophyceae		
Amphidinium carterae Hulburt	Guillard, clone "Amphi 1"	Good growth[b]
Chrysophyceae		
Olisthodiscus luteus Carter	Conover, clone "Olisth"	Good growth[f]
Bangiophyceae		
Porphyridium cruentum Naegeli	Vischer strain No. 107	Good growth[e]

[a]Droop (1955).
[b]Antia et al. (1975).
[c]Antia et al. (1977).
[d]Conover (1975).
[e]McCarthy (1971).
[f]Mahoney and McLaughlin (1977).

Materials and Methods

Algal Species

Stock cultures of the algae listed in Table 1 were routinely maintained on nitrate (as nitrogen source), under standard axenic conditions, according to Antia and Cheng (1970).

Growth Tests

All tests were carried out in culture tubes at 18°C under continuous illumination (irradiance 95–100 $\mu E \cdot m^{-2} \cdot s^{-1}$) from cool-white fluorescent lamps. The standard test medium was that of Oliveira and Antia (1984) with the salinity adjusted to 26‰. In the case of *Olisthodiscus luteus* and *Cyclotella cryptica*, final salinity of the test medium was retained at 14‰. Unless otherwise stated, Ni^{2+}, nitrate, and urea additions were made to 4-mL aliquots of test media before inoculation with 0.2-mL aliquots of the algal stock culture. All test media were filter-sterilized (0.2-μm pore size presterilized Nalgene filters) and aseptic techniques were used throughout the tests to ensure axenic growth conditions.

Growth was monitored directly in culture tubes by periodically reading their optical density at 600 nm on a spectrophotometer after brief vortex mixing. Extended periods of incubation (up to 60 d) were allowed for cases of delayed algal adaptation in order to avoid some of the experimental pitfalls pointed out by Stockner and Antia (1976). Every growth test included a control without any added nitrogen source, and the growth measured on a nitrogen source was corrected by subtracting that measured on the control. Plots of the corrected growth against incubation period were used to calculate the following three parameters: (1) adaptation period = number of days from inoculation to the first significant increase in OD_{600}, (2) exponential growth rate = maximal increase in OD_{600} per day during exponential phase of growth, expressed as percentage of corresponding rate from nitrate control, and (3) maximum yield = maximal OD_{600} on the growth curve for a test, expressed as percentage of similar yield from growth on nitrate. The nitrogen stoichiometry for equivalent growth was defined by the equation 1 m*M* urea ≡ 2 m*M* nitrate (Oliveira and Antia 1984).

Results

All 12 species tested were able to utilize urea as the sole source of nitrogen (Fig. 1, 2, and 3). Six of these species showed greater cell yields from growth on urea compared with those from equivalent nitrate. Particularly noticeable in this respect were the growth responses of *Pavlova lutheri* (Fig. 1A), *Chaetoceros gracilis* (Fig. 1D), and *Rhodomonas* sp. (Fig. 3A), which showed 50% or greater maximal yield on urea relative to the nitrate controls.

All tested species showed significant growth improvement with increasing urea concentration in the absence of nickel supplementation (Table 2). Most of the algae displayed the highest values for growth rate and maximum yield when grown

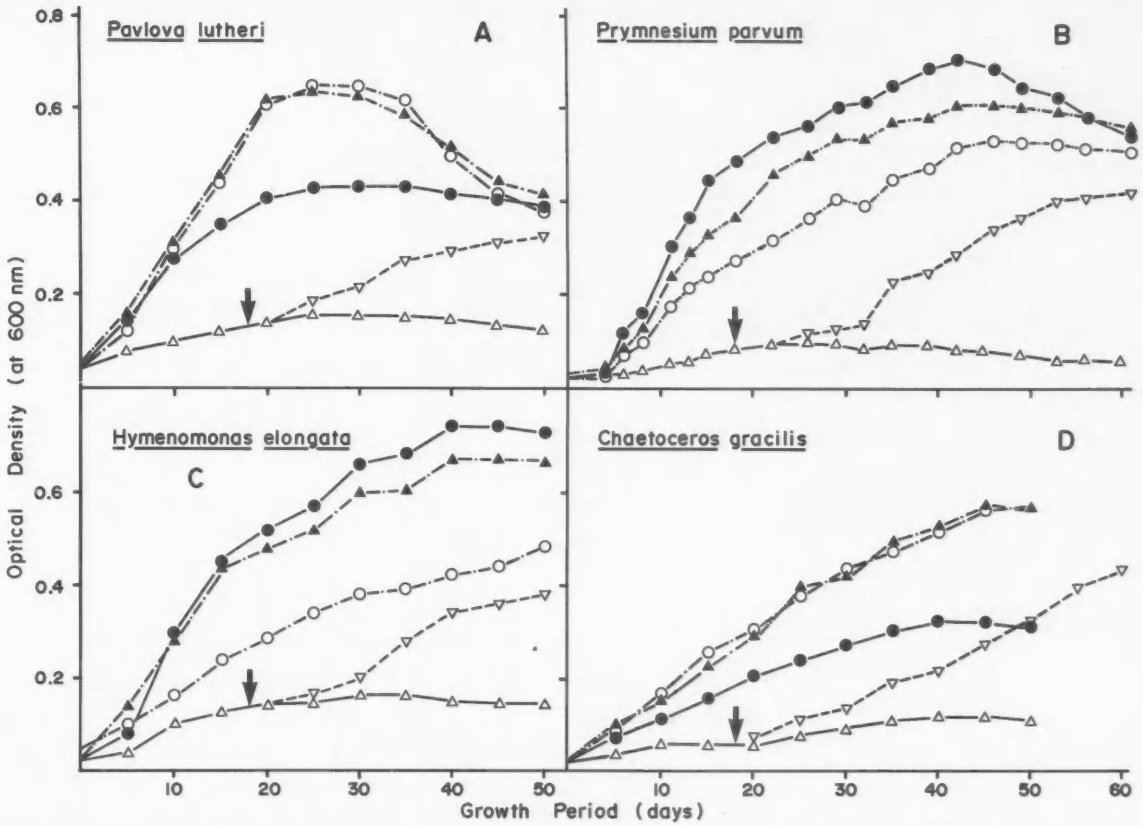

FIG. 1. Growth curves on urea (1 m*M*) without Ni^{2+} addition (○), with 0.1 μ*M* Ni^{2+} (▲), with 5 m*M* citrate (△), and with 5 m*M* citrate followed by the addition of 25 μ*M* Ni^{2+} (▽) at day 18 (↓). Corresponding control growth on nitrate (2 m*M*) without Ni^{2+} and citrate addition is shown (●).

TABLE 2. Growth of microalgae on urea without nickel supplementation. *a*, adaptation period; *b*, exponential growth rate; *c*, maximum yield; see text for details.

	Urea concentration (m*M*) in growth medium											
	0.25			1.00			5.00			10.00		
Alga	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)
Rhodomonas sp.	0	102	124	0	156	196	0	165	245	0	112	132
Olisthodiscus luteus	12	40	111	10	56	146	6	84	184	14	44	96
Amphidinium carterae	0	94	86	0	134	130	0	130	178	3	110	98
Pavlova lutheri	0	110	92	0	146	150	0	144	146	0	108	102
Chaetoceros gracilis	2	72	68	0	142	160	0	126	144	0	96	112
Thalassiosira nordenskioldii	0	45	72	0	80	118	0	86	126	0	56	86
Achnanthes brevipes	16	88	74	15	104	100	12	122	114	16	72	68
Skeletonema costatum	0	83	92	0	124	118	2	108	98	4	87	82
Thalassiosira weissflogii	2	58	74	0	112	106	0	118	108	3	54	68
Porphyridium cruentum	6	22	32	4	32	70	2	54	108	0	72	136
Prymnesium parvum	6	56	48	4	65	68	1	92	102	2	60	62
Hymenomonas elongata	3	22	18	0	64	42	0	66	78	2	42	44

with 5 m*M* urea, although the differences in magnitude for this and the 1 m*M* concentration were generally small. The most noticeable exceptions were *Amphidinium carterae*, *Prymnesium parvum*, and *Hymenomonas elongata* which showed an improvement of 50% or more on their cell yields when cultured with 5 m*M* urea. There is little correlation, however, between improvement of ultimate yield and exponential growth rate values at this concentration compared with those at 1 m*M*.

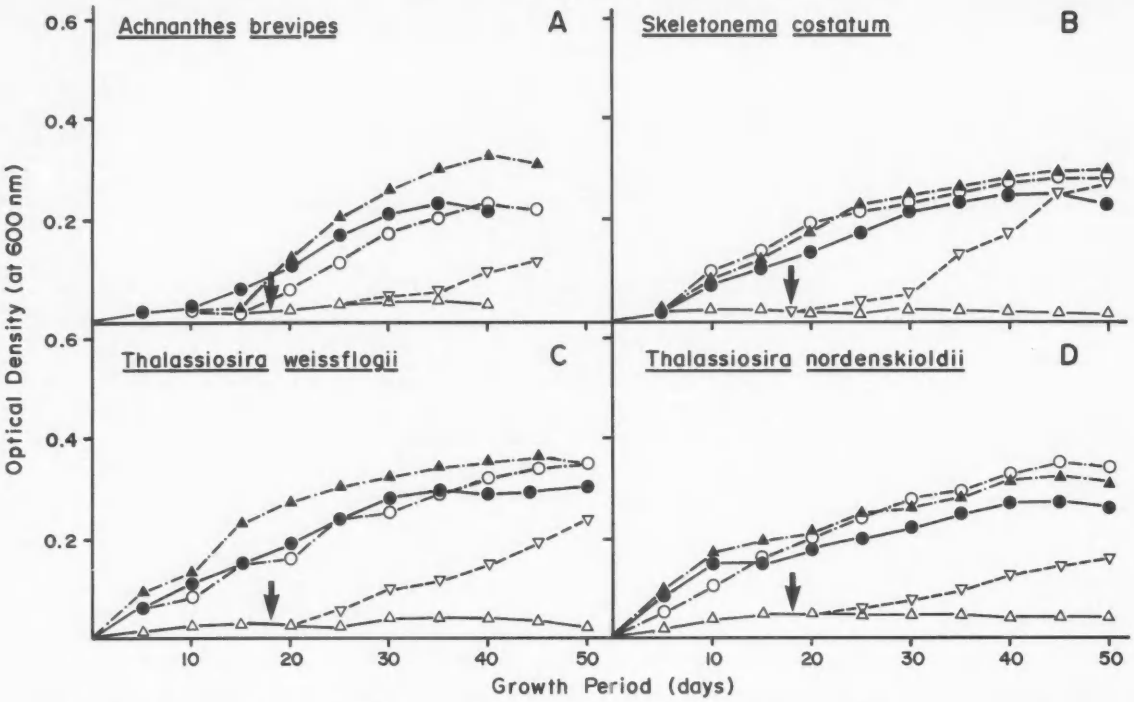

FIG. 2. Growth curves on urea (1 m*M*) without Ni^{2+} addition (○), with 0.1 μ*M* Ni^{2+} (▲), with 5 m*M* citrate (△), and with 5 m*M* citrate followed by the addition of 25 μ*M* Ni^{2+} (▽) at day 18 (↓). Corresponding control growth on nitrate (2 m*M*) without Ni^{2+} and citrate addition is shown (●).

TABLE 3. Growth of microalgae on urea (1.0 m*M*) with nickel supplementation. *a*, adaptation period; *b*, exponential growth rate; *c*, maximum yield; see text for detais (nm = values too low or inadequate for measurement with confidence).

	Nickel supplementation (μ*M*)														
	5×10^{-3}			1×10^{-2}			1×10^{-1}			1			10		
Alga	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)	*a* (days)	*b* (%)	*c* (%)
Rhodomonas sp.	0	102	136	0	128	195	0	110	186	0	108	125	7	36	47
Achnanthes brevipes	16	130	122	14	176	150	15	156	146	16	140	134	nm	nm	nm
Amphidinium carterae	0	160	138	0	182	146	0	148	136	4	108	102	9	26	27
Thalassiosira weissflogii	0	110	108	0	132	140	0	130	126	0	102	100	6	20	28
Skeletonema costatum	0	132	126	0	130	136	0	126	120	6	88	78	12	26	38
Thalassiosira nordenskioldii	0	100	98	0	106	116	0	106	128	2	76	92	8	30	32
Pavlova lutheri	0	106	112	0	104	108	0	100	100	0	96	96	8	26	36
Chaetoceros gracilis	0	106	112	0	102	108	0	96	100	8	69	89	22	14	29
Hymenomonas elongata	0	80	82	0	100	102	0	90	92	2	60	64	8	18	20
Porphyridium cruentum	4	56	88	4	80	96	4	80	86	6	26	40	20	10	16
Prymnesium parvum	0	104	108	0	100	90	2	94	88	2	76	64	nm	nm	nm
Olisthodiscus luteus	20	24	36	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm

Porphyridium cruentum showed continuous improvement in both growth rate and maximum yield values with increasing amounts of urea supplementation, the highest values for these two parameters being recorded at the highest concentration tested (10 m*M*). In this alga, increased efficiency in urea utilization is accompanied by a progressive decrease in the adaptation lag period which is eliminated at the highest urea concentration tested. *Achnanthes brevipes* and *Olisthodiscus luteus* required long adaptation lag periods for all urea concentrations tested before growth occurred on this nitrogen source. In the case of the other algae, very little or no lag period was required, irrespective of the urea concentration used.

Supplementation of the culture medium with 0.1 μ*M* Ni scarcely improved the growth response of the species tested (Fig. 1, 2, and 3), apart from the cases of *Prymnesium parvum* (Fig. 1B), *Hymenomonas elongata* (Fig. 1C), and *Achnantes brevipes* (Fig. 2A). *Thalassiosira nordenskioldii*, while not displaying much improvement, nevertheless showed optimal

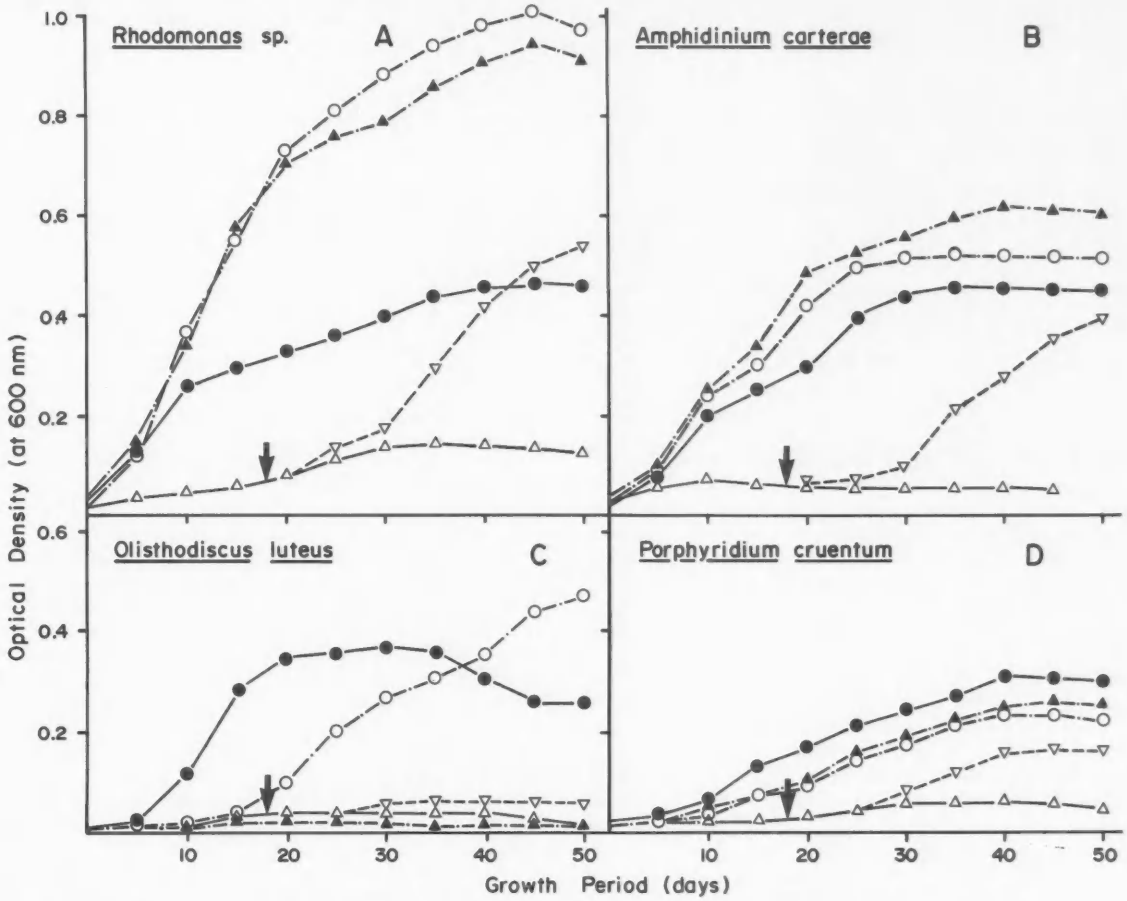

FIG. 3. Growth curves on urea (1 m*M*) without Ni^{2+} addition (○), with 0.1 μ*M* Ni^{2+} (▲), with 5 m*M* citrate (△), and with 5 m*M* citrate followed by the addition of 25 μ*M* Ni^{2+} (▽) at day 18 (↓). Corresponding control growth on nitrate (2 m*M*) without Ni^{2+} and citrate addition is shown (●).

TABLE 4. Optimal nickel supplementation for growth of microalgae on urea (1 m*M*). Growth parameters are expressed as percentage of those in media containing nitrate (2 m*M*).

Alga	Nickel (n*M*)	Growth rate[a]	Maximum yield[a]
Rhodomonas sp.	10	128 (156)	195 (196)
Achnanthes brevipes	10	176 (104)	150 (100)
Amphidinium carterae	10	182 (134)	146 (130)
Thalassiosira weissflogii	10	132 (112)	140 (106)
Skeletonema costatum	10	130 (124)	136 (118)
Thalassiosira nordenskioldii	100	106 (80)	128 (118)
Pavlova lutheri	5[b]	106 (146)	112 (150)
Chaetoceros gracilis	5[b]	106 (142)	112 (160)
Prymnesium parvum	5	104 (65)	108 (68)
Hymenomonas elongata	10	100 (64)	102 (42)
Porphyridium cruentum	10	80 (32)	96 (70)
Olisthodiscus luteus	5[b]	24 (56)	36 (146)

[a]Values in parentheses are for urea (1 m*M*) supported growth in the absence of nickel supplementation.

[b]Owing to the toxicity of nickel at all concentrations tested, the optimal Ni^{2+} concentration, in these cases, is expected to be lower than that shown, if nickel is at all required for growth on urea.

values for both growth rate and cell yield when supplemented with 0.1 μ*M* Ni (Table 3). All the other algae required lower amounts of nickel for optimal growth as measured by those two parameters (Table 4). The highest tested nickel concentration (10 μ*M*) proved to be severely deleterious to the growth of all the microalgae, while the lowest tested concentration (5 n*M*) gave rise to suboptimal growth for the majority of the algae (Table 3). *Olisthodiscus luteus* proved to be extremely sensitive to nickel supplementation, showing growth inhibition even at the lowest tested concentration (Table 3). *Chaetoceros gracilis* and *Pavlova lutheri* also proved to be growth inhibited (although not to the same extent) at all nickel concentrations tested in showing growth rate and yield values significantly below those observed in urea-grown cultures without nickel supplementation (compare Tables 2 and 3).

A clear dependency on nickel for growth on urea could be demonstrated by growing the microalgae in the presence of a nickel chelator such as citrate. Since addition of citrate had little effect on the growth from nitrate, the observed inhibition of urea growth from the presence of citrate could not be attributed to toxicity from citrate. Citrate inhibition of urea growth could be annulled for all microalgae tested, with the exception of *Olisthodiscus luteus* (Fig. 3C), by the addition of an excess of nickel to the citrate-supplemented cultures (Fig. 1, 2, and 3).

Discussion

The results clearly demonstrate the widespread occurrence of urea-utilizing capabilities on the part of all microalgae tested, with significantly greater growth by some species relative to the nitrate controls. Even those algae previously reported to show poor or no growth on urea, such as *Hymenomonas elongata* (Droop 1955), *Prymnesium parvum* (Droop 1955), and *Thalassiosira nordenskioldii* (Conover 1975), showed excellent growth under our test conditions. We used the same clones as the previous investigators, except for *Thalassiosira nordenskioldii*; therefore, we attribute the discrepancies to differences in culture conditions or media composition.

The results leave no doubt that virtually all the algae require nickel for growth on urea, although this requirement is satisfied in most cases at very low nickel concentrations. Since the endogenous nickel content of the seawater used in the preparation of our test media is 3.42 n*M* and our estimate of endogenous nickel in the total medium is of the order of 2.7 and 1.4 n*M* for media of salinity 26 and 14‰, respectively (Oliveira and Antia 1984), the nickel concentrations required for optimal growth of most of the tested algae lie in the range of 5–15 n*M*, barring special cases such as those of *Cyclotella cryptica* (Oliveira and Antia 1984) and *Thalassiosira nordenskioldii* (Table 4) which seem to require unusually high levels of nickel. In the case of *Chaetoceros gracilis* and *Pavlova lutheri*, the low endogenous nickel concentration (2.7 n*M*) of the culture medium is sufficient to support excellent urea-dependent growth, since nickel supplementation partially suppresses growth even at the lowest concentration added (5 n*M*). This may also be the case for *Olisthodiscus luteus*, although the greater sensitivity of this alga to inhibition from nickel additions makes the results inconclusive.

Several investigators determined nickel concentrations in ocean water from various regions of the world and found values ranging from approximately 1.7 to 10.4 n*M*, with the mean values in the order of 3.5–5.3 n*M* for surface waters (Danielsson 1980; Danielsson et al. 1985; Jones and Murray 1984; Sclater et al. 1976; Yeats and Campbell 1983). While some of these oceanic nickel concentrations might be too low to support fair-to-good growth of some species of microalgae on urea, other concentrations seem sufficient to support an effective utilization of urea on the part of most urease-producing algae.

An inference drawn from these studies is the realization that growth parameters obtained in natural seawater of unknown and variable endogenous nickel content are unsatisfactory and unreliable indicators of a nickel requirement for the growth of microalgae with urea as nitrogen source (compare Tables 2 and 3). However, the citrate-dependent observations support the validity of nickel requirement where citrate, as shown by Kersten et al. (1980), functions as an excellent Ni^{2+} chelator. The Ni^{2+}-induced reversal of citrate inhibition observed for virtually all the tested algae further confirms our view of a universal nickel requirement for urea utilization by the urease-producing species. Nevertheless, careful consideration of the relevant literature suggests a couple of objections to this conclusion that should be considered here.

Jackson and Morgan (1978) showed that additions of very high concentrations of a chelator to the growth medium decreased the growth rate of phytoplankters.This overchelation response could not be readily associated with any one of the metal ions studied by these investigators, but instead it seemed to be related to the available concentrations of one or more essential trace metal ions which became too low to support growth. According to this viewpoint, the citrate inhibition of urea-grown cells could be attributed to a reduction in the availability of trace metal ions other than Ni^{2+}. Furthermore, Harrison and Morel (1983) showed that the addition of only one metal ion to the growth medium could result in an increase in the free ion concentration of other metals by displacing them from combination with the chelator. Thus, it would appear that additions of nickel to citrate-inhibited urea-supplemented cultures could promote the restoration of growth by making some other trace metal ion(s) available to the microalgae. These viewpoints cannot be completely excluded by the results obtained in the present investigation. However, we must point out that in the present study, the growth inhibition response from citrate addition was restricted to urea-grown cultures, since it was not observed from the addition of citrate to nitrate-grown cells. The citrate-induced growth inhibition could also be overcome, to varying degrees depending on the microalga tested, by the addition of excess nickel or nitrate without nickel (unpublished results) to urea-grown cells. These observations clearly indicated that the trace metal ions normally required for growth on nitrate were not removed by the citrate addition, while the reversal by nickel of citrate inhibition appeared more specific than mere displacement of other trace metal ions from chelation. It seems, therefore, that the restoration of growth on citrate-inhibited urea-supplemented cultures is, at least partially, a function of Ni^{2+} availability rather than overchelation of one or more essential trace metal ions or of an increase in the free ion concentration of other metals in the growth medium due to the addition of excess Ni^{2+}. Further work is now in progress to elucidate the specific nature of Ni^{2+} requirement in urea utilization by microalgae.

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Family Size and Effective Population Size in a Hatchery Stock of Coho Salmon (*Oncorhynchus kisutch*)

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Simon, R. C., J. D. McIntyre, and A. R. Hemmingsen. 1986. Family size and effective population size in a hatchery stock of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 43: 2434–2442.

Means and variances of family size measured in five year-classes of wire-tagged coho salmon (*Oncorhynchus kisutch*) were linearly related. Population effective size was calculated by using estimated means and variances of family size in a 25-yr data set. Although numbers of age 3 adults returning to the hatchery appeared to be large enough to avoid inbreeding problems (the 25-yr mean exceeded 4500), the numbers actually contributing to the hatchery production may be too low. Several strategies are proposed to correct the problem perceived. Argument is given to support the contention that the problem of effective size is fairly general and is not confined to the present study population.

Les moyennes et les variances de la taille des familles de cinq classes annuelles de saumons cohos (*Oncorhynchus kisutch*) marqués par fils codés présentaient une relation linéraire. La taille effective de la population a été calculée à partir des moyennes et des variances estimées de taille de famille tirées d'un ensemble de données portant sur 25 ans. Bien que le nombre d'adultes d'âge 3 revenant aux installations piscicoles semblait suffisamment important pour éviter les problèmes de consanguinité (la moyenne sur 25 ans était supérieure à 4 500), le nombre de poissons contribuant réellement à la production piscicole pourrait être trop faible. Les auteurs proposent diverses stratégies pour corriger ce problème. Ils présentent aussi des arguments appuyant l'hypothèse que le problème de la détermination de la taille effective est relativement généralisé et non limité à la population étudiée.

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In the sense of a population's breeding history, effective population size (N_e) may be estimated from Wright's (1939) expression for obtaining the harmonic mean of census numbers in (t) successive generations, beginning with some starting number (N_1) and ending with N_t):

$$(1)\quad N_e = [1/t(1/N_1 + 1/N_2 + \ldots + 1/N_t)]^{-1}.$$

From a few trial numbers (e.g. $N_1 = 10\,000$, $N_2 = 500$, $N_3 = 5$, $N_t = 10\,000$; then $N_e \sim 20$), it is seen that N_e is much nearer the smaller breeding number than to the larger. The significance of this (rather extreme) result is that present census numbers do not necessarily guarantee that the genetic signature of the population is closely similar to that existing earlier, especially when N_t, or some N_{t-i}, is small.

Such restrictions in breeding number, if pronounced, are virtually certain to result in the scrambling of allele frequencies that represented (in total) an approach toward stability under prevailing environmental circumstances.

Published work by many authors has shown that breeding structure of populations further influences N_e in aspects additional to numbers of parents. Population breeding structure includes several important factors, such as parental sex ratio, degree of generation overlap, number of successful lifetime matings, extent of polygamy, presence of assortative mating, and number of progeny per mating that survive to reproduce successfully. When several of these population parameters vary simultaneously, the task of sorting out the major implements of genetic change may become formidable, or at least evasive, unless rather extensive simplifying assumptions are applied. Fortunately, in some species, such as the coho salmon (*Oncorhynchus kisutch*), the need for simplifying assumptions is reduced because many of these factors are rather clearly defined. Hatchery stocks in particular are good models of numerical and other genetic factors because accurate records are available on sex ratio, age at maturity, numbers of adults returning, numbers of smolts released, and details of mating procedures. For example, the presence of assortative mating (deliberate exclusion of age 2 males from the sperm pool) ensures that overlap of generations is minimal or absent. This practice effectively specifies the existence of three different

lines within the hatchery, each of which is genetically separated. The practice also reduces the number of males contributing to the next generation, requiring additional adjustment of N_e to account for the unequal numbers of female (N_f) and male (N_m) parents. Further, the invariable fact of a single maturation in a lifetime removes yet another difficulty, as does artificial fertilization, thus assuring participation of rather accurately known numbers of male and female parents. Also, because the extent of straying between stocks is low (or at least sometimes definable), the factor of migration cannot often be a major source of confusion. Finally, because some tagging studies have been done in which progeny of individual matings have been marked (as in the present study), it has been possible to define family size (the number of progeny from each mating that survive to reproduce) and to examine the effects of these numbers on N_e. Consequently, the objective of our study was to define, to the extent possible, the significance of the factors mentioned above in the context of population effective size.

Materials and Methods

Hatchery Location and Spawning Operation

Big Creek Hatchery is near Knappa, Oregon, on Big Creek, about 6.4 km from its confluence with the Columbia River. The confluence is about 97 km upstream from the Pacific Ocean.

The spawning operation typically begins near the peak of the returning run of adult coho salmon (about November 7) and continues intensively for a few days. In general, males approaching age 2 (hereafter termed 1+) are rigorously excluded from matings in favor of 2+ males approaching their third year (females mature exclusively at age 2+ in the Big Creek population). Matings are made by releasing eggs from three to five females, by abdominal incision, into a plastic bucket, or similar container. Milt from one to three males is then added and is typically mixed before eggs from three to five additional females are added, again followed by milt from one to three males. Males may or may not be used repeatedly, but often are because females are the more numerous. Fertilized eggs are allowed to stand about 10 min; they are then washed with creek water by filling the container and decanting. After the washed eggs are water hardened, they are placed in incubator trays, or baskets. This process is repeated until all needed or available eggs have been fertilized.

When eye pigmentation in embryos is strongly evident, embryos not required for in-house smolt production are shipped for use elsewhere. Smoltification typically takes place in May, some 18 mo postfertilization, and 1+ males return in November of the same year. Mature 2+ fish return 1 yr later.

Tagging with Coded Wire

More than 400 000 fingerlings from a total of 158 families (a family is defined as the offspring (brothers and sisters) of one female) from the 1967, 1968, 1971, 1974, and 1977 year-classes were distinctively marked by using equipment and techniques described by Jefferts et al. (1963) and Bergman et al. (1968). The adipose fin was also removed from each tagged fish. Eggs of each female were fertilized separately and were reared separately until the resulting fingerlings were tagged (about 6 mo postfertilization). Mortality was recorded from the beginning of incubation to the time of tagging; families were then mixed in raceways. Thereafter, mortalities were recorded daily and assigned to the appropriate family according to tag code.

Fecundity Estimates

Eggs from each of the 158 females were drained of ovarian fluid and water after fertilization and then weighed. Eggs in a weighed sample from each female were counted, and the egg total number was estimated from the sample count and weight. Sample counts were assisted by using a plastic plate containing 100 drilled depressions of a diameter sufficient to hold only one egg each. About one fifth of the total egg volume was counted for each female.

Estimation of Parental Numbers Contributing to Smolt Release

Although numbers of fish of each sex returning to the hatchery are accurately and routinely recorded, the numbers contributing to the smolt release (constituting the next generation) are not because many (sometimes most) of the eggs taken are transfered elsewhere (i.e. are exported in the sense that they will not contribute further to the in-house population). It is important to note that exports are made by removing the contents of whole trays (or baskets, both of which were used) and not by pooling of small samples from many containers. Thus, exports nearly always represent the entire egg take from several females, and the eggs retained similarly represent the entire take from other females. We hence have estimated the number of female parents (N_f) contributing to the smolt release as being the number of smolts released divided by the mean number surviving to smolt per family. The number of male parents potentially contributing to the smolt release was calculated from the male to female (m:f) sex ratio observed three year-classes earlier than the smolt year-class from $N_m = N_f \times (\text{m:f})$. Because m:f ratios were not available for the three year-classes preceding 1957, we substituted the mean ratio determined from the 25-yr data set. We thus assumed that the eggs in trays designated for smolt production in-house were fertilized (at maximum) by the same proportion of males as was observed in the returning adults. It was further assumed that exports were made before the eggs hatched because the shipment of small fish is relatively difficult, and mortality and disease risks are increased. Although the transfer of eggs rather than fry is not an invariable practice, in general the transfer of small fish is discouraged.

Enumeration of Smolts Released and Adult Returns

Returning adults were individually counted as they were removed from the trap and were segregated by sex into separate pens. Numbers of smolts released were estimated from the average individual weight (obtained by counting a weighed sample) and the total weight of all smolts released.

Means and Variances of Family Size

In the general (unmarked) hatchery return, maternal mean family size was estimated by dividing the number of adult returns by the number of females that produced them. Paternal mean family size was estimated by dividing maternal family size by the m:f sex ratio. For the wire-tagged groups, maternal family size was measured by direct count of coded tags. Regression of the family size variances in the wire-tagged groups on their respective means produced a regression equation that was used to estimate the variance of family size for the un-

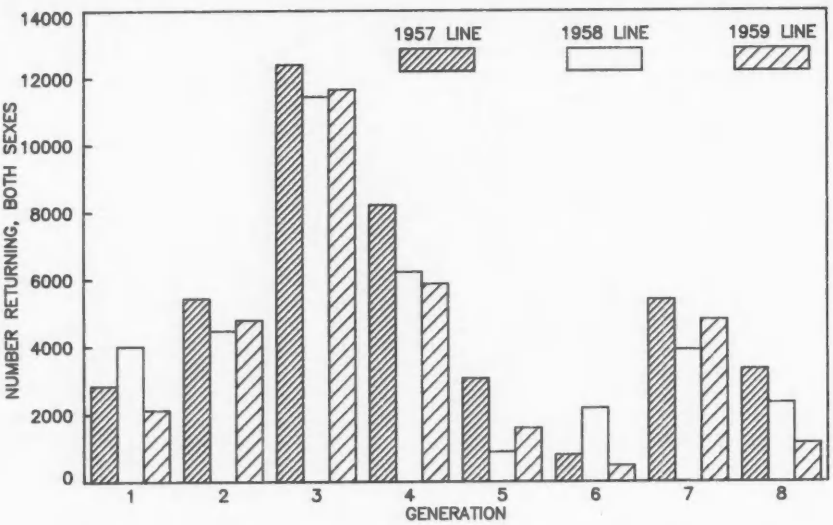

FIG. 1. Returns, by generation, of 2+ fish for three lines of Big Creek Coho salmon.

marked hatchery returns. We assumed that the variance of paternal family size was also adequately estimated from the maternal regression and calculated the paternal variances accordingly.

Estimation of Parental Effective Numbers

From a very general application of the Kolmogorov forward (Fokker–Planck) equation (Kolmogorov 1935; Wright 1945), Crow and Morton (1955) derived an expression for the variance of change in frequency of genes in females from diploid populations with separate sexes:

$$(2)\quad V_{dgf} = \frac{(g_f(1 - g_f))}{2N_T}\,[(1 - F_f + (1 + F_f)V_{kf}/u_{kf}]$$

where N_T is the number of adults of both sexes, F is Wright's inbreeding coefficient (Wright 1921, 1922), V_{kf} is the variance in family size of female parents, u_{kf} is the maternal mean family size, and g_f is the female allelic frequency. The expression is independent of the actual form of the family size distribution. The term V_{dgm} is of the same form, with appropriate subscript changes. By analogy to equations 2 and 3 of Crow and Morton, the effective female number may be written

$$(3)\quad N_{ef} = \frac{N_T}{[1 - F_f + (1 + F_f)V_{kf}/u_{kf}]}.$$

This expression applies when the numbers of males and females are equal; however, when this is not true (a point not expanded on by Crow and Morton), the expression requires modification to the form

$$(4)\quad N_{ef} = \frac{2N_f}{[1 - F_f + (1 + F_f)V_{kf}/u_{kf}]}.$$

The same expression, with appropriate subscript changes, applies to the paternal effective number:

$$(5)\quad N_{em} = \frac{2N_m}{[1 - F_m + (1 + F_m)V_{km}/u_{km}]}.$$

A restriction applies to equations 4 and 5 in that V_{kx}/u_{kx} is not <1, to avoid the dubious conclusion that the effective number is larger than the census number, as it would be when $F = 0$.

When, as in the present data, the numbers of males and females are unequal, Wright's (1931) correction is applied by using N_{ef} and N_{em} from equations (4) and (5) in the equation

$$(6)\quad N_e = 4N_{ef}N_{em}/(N_{ef} + N_{em})$$

to define the total effective number consisting of equal numbers of each sex. Three models were applied to the N_e data obtained by using the above equations. The first assumed that $F = 0$ in both sexes and that all available 2+ adults were mated; the second assumed that $F = 0.3$ and that all available 2+ adults were mated; and the third assumed that all available females were mated, that half of available males participated in matings, and that $F = 0.3$.

General Statistical Procedures and Historical Data

Correlations and partial correlations were calculated between N_e and other population data (percent survival of 2+ and 1+ fish, and numbers of smolts released) according to standard methods. Regression of 2+ survival to the hatchery on N_e was based on model II assumptions (bivariate normal distribution).

The 25-yr data set was obtained from archival records of the Oregon Department of Fish and Wildlife. Because sex data from the 1975–80 year-classes were either missing or suspect (e.g. number of males near or greater than number of females), we either used earlier data exclusively or first analyzed the earlier data separately before analyzing the earlier and later data combined. In the regression analysis of percent survival of 2+ fish to the hatchery on $N_{e'}$, the $N_{e'}$ values came from the parental generation three year-classes earlier; thus, only 22 yr of the 25-yr data set could be used.

Similarly, when calculating the proportion of females used for in-house smolt production, we used the total number available from the parental generation three year-classes earlier than that of the smolt release from which the number of females actually used was estimated. For example, the proportion of available females contributing to the 1960 year-class (YC) smolt release was

TABLE 1. Population data for coho salmon, Big Creek Hatchery, 1960–84.

Year-class	No. of smolts released	No. of 2+ adults returned	Survival (%)	m:f ratio	Mean family size[a]	
					Maternal	Paternal
1957	940 397	2 820	0.30	0.68	7.32	10.76
1958	690 772	4 003	0.58	0.65	14.13	21.74
1959	1 024 554	2 121	0.21	0.54	5.05	9.35
1960	1 191 890	5 432	0.46	0.67	11.12	16.60
1961	1 280 726	4 466	0.35	0.68	8.50	12.50
1962	1 965 094	4 788	0.24	0.64	5.94	9.28
1963	1 832 810	12 390	0.68	0.62	16.49	26.60
1964	1 379 810	11 444	0.83	0.62	20.23	32.63
1965	1 527 366	11 659	0.76	0.81	18.62	22.99
1966	1 291 645	8 213	0.64	0.74	15.51	20.96
1967	700 026	6 221	0.89	0.59	21.67	36.73
1968	665 623	5 864	0.88	0.71	21.49	30.27
1969	851 295	3 051	0.36	0.77	8.74	11.35
1970	759 100	883	0.12	0.50	2.84	5.68
1971	724 621	1 586	0.22	0.75	5.34	7.12
1972	858 800	792	0.09	0.56	2.25	4.02
1973	790 999	2 176	0.28	0.69	6.71	9.72
1974	875 870	476	0.05	0.76	1.33[b]	1.75[b]
1975	775 258	5 388	0.69	0.68[c]	16.95	25.68
1976	429 371	3 897	0.91	0.68	22.14	33.55
1977	534 420	4 797	0.90	0.68	21.89	33.17
1978	575 537	3 324	0.58	0.68	14.09	21.35
1979	499 736	2 336	0.47	0.68	11.40	17.27
1980	700 041	1 136	0.16	0.68	3.96	6.00
1981	955 938	5 950	0.62	0.92	7.90	11.97

[a] Where maternal mean family size = 2+ returns ÷ (smolts ÷ 2439) = (mmfs) and paternal mean family size = (mmfs) ÷ (male:female).
[b] $V_k/u_k < 1$; actual ratio used from Table 4 for N_e estimates (Table 2).
[c] 1975–80 sex data incomplete; mean of other years substituted.

$$\frac{\text{smolts released}/2439}{\text{1957 YC adults}/(1 + \text{m:f sex ratio})} = \frac{\text{number contributing}}{\text{number available}}$$

$$= 0.291$$

for which only 22 data pairs are available. (For convenience, some of the above computational details are repeated in the pertinent tabular portions of the results section.)

In small populations there is a tendency for heterozygosity to decrease (due to an inbreeding effect) and for gene frequencies to drift randomly due to nonrepresentative sampling (the variance effect). The term N_e is calculated differently, depending on which effect is of interest (e.g. Crow and Kimura 1970). In either situation, a small N_e may be viewed as a measure of impending genetic destabilization. In the strict sense, equations 2–6, on which our N_e values are based, apply to the variance effective size.

Results

Average fecundity for Big Creek coho was estimated to be 3024 eggs per female, and the mean number surviving from the egg to smolt stage was 2439. Numbers of 2+ adults returning to the hatchery peaked in 1963 and have generally declined thereafter (Fig. 1). Summary data on smolts released and adults returned are given in Table 1. From outward appearances, fully adequate numbers of adults were available (averaging 4609) to allow only slight drift or inbreeding. However, when the egg allocations for smolt production and for export are taken into account (Table 2), a much less satisfactory view of the hatchery's operating efficiency is evident.

Calculated maternal family size data from Table 1, as summarized in Table 3, appear to fit a uniform (or rectangular) distribution. The observed distributions of family size for wire-tagged groups (Table 4) indicate that the distributions of 1967 and 1977 year-classes are quasi-Poisson, or else resemble negative binomial distributions. None of the observed distributions fit the above distribution models satisfactorily, partly because of extreme outliers, suggesting that the true distributions are probably mixtures of different distributions, or that the samples are not adequate for correct assessment. In any event, the survival of fish of different families appears to be nonrandom in some years.

The three lines of Big Creek coho salmon tended to be similar in number when compared by generation, provided that generation 1 is started in the "right" year (Fig. 1). Had the first line been set to generation 1 in the 1958 or 1959 year-classes, for example, the agreement within generation would be very different. Over the eight generation sets-of-three observed, the

TABLE 2. Calculated actual and effective numbers of coho salmon produced in 25 spawning years. Symbols: (a) N_f and N_m = calculated breeding numbers of females (f) and males (m); (b) N_{ef} and N_{em} = effective numbers of females and males calculated from equations 4 and 5, assuming $F = 0$ and that the census numbers of both sexes were mated; (c) $N_{ef'}$ and $N_{em'}$ = effective numbers as in (b), assuming $F = 0.3$ and that census numbers of both sexes were mated; (d) N_e = effective number of combined sexes when (b) values are used in equation 6 and $N_{em''} = 0.5N_{em'}$; (e) $N_{e''}$ = effective number of combined sexes as in (d), but assuming that one half of available males and all available females were mated, using $N_{ef'}$ and $N_{em''}$ in equation 6.

	a		b		c		d		e
Year-class	N_f	N_m	N_{ef}	N_{em}	$N_{ef'}$	$N_{em'}$	$N_{em''}$	N_e	$N_{e''}$
1957	386	262	189	120	163	103	51	294	155
1958	283	192	125	82	107	70	35	198	105
1959	420	286	227	133	200	115	58	335	180
1960	489	333	222	145	191	124	62	351	187
1961	525	341	248	153	204	131	66	379	201
1962	806	435	415	203	361	175	88	545	282
1963	751	503	327	212	280	181	90	515	273
1964	566	385	242	161	207	137	68	387	206
1965	626	401	269	170	231	145	73	418	221
1966	530	329	232	141	199	120	60	350	185
1967	287	178	122	74	104	63	31	184	97
1968	273	221	116	93	99	79	39	206	113
1969	349	258	164	117	142	100	50	273	148
1970	311	183	232	95	215	83	42	270	140
1971	297	211	158	104	138	90	45	250	136
1972	352	271	339	161	335	144	72	437	237
1973	326	163	162	76	141	65	33	206	106
1974	359	269	203	152	179	134	67	347	195
1975	318	178	138	75	118	64	32	194	101
1976	176	121	75	50	64	43	21	121	64
1977	219	166	93	69	80	59	29	159	86
1978	236	156	104	67	89	57	28	163	86
1979	205	135	93	58	80	50	25	144	76
1980	287	189	172	97	154	85	42	248	111
1981	392	267	188	120	163	103	52	294	157

TABLE 3. Maternal family size, 1960–84: chi-square goodness-of-fit for uniform distribution (chi-square = 4.16; df = 8; $p \simeq 0.8$).

Cell No.	Lower limit	Number observed	Number expected
1	0.0	2	2.78
2	2.5	3	2.78
3	5.0	4	2.78
4	7.5	3	2.78
5	10.0	2	2.78
6	12.5	2	2.78
7	15.0	3	2.78
8	17.5	1	2.78
9	20.0	5	2.78

correlations of 2+ numbers between the 1957, 1958, and 1959 lines were as follows: $r_{57,58} = 0.93$, $r_{58,59} = 0.96$, and $r_{57,59} = 0.98$; the corresponding slopes were 0.832, 1.067, and 0.981 and the intercepts were 124.2, −673.4, and 1,199.0. Thus, from the 1957 line returns in 1984 (1981 year-class) the 2+ return prediction would be about 7000 in November 1986, and so forth. Other data in Table 5 further suggest that 1+ returns may also be fair estimators of 2+ male returns in the following year, especially if N_e is taken into account.

Significant associations between $N_{e''}$ and survival from smolt to age 2+ were also observed. The number of smolts released was not significantly associated with percent survival, but apparently did influence the number of males returning (Table 5). Regression estimates of percent survival on $N_{e''}$ (Table 6) suggest substantial opportunity to increase survival to the hatchery, and hence to also increase the ocean catch potential, by increasing N_e. It is clear that N_e could have been very substantially increased in most previous years (Table 7), and further opportunity for future increases is indicated.

Discussion

Because appreciable correlation was observed (approaching $p \leq 0.001$) between $N_{e''}$ (also N_e, since the two are proportional) in the parent generations and 2+ survival in the progeny generations, we suspect that even our most pessimistic model may be too optimistic. Even though $N_{e''}$ was observed to be small in some years, it did not appear to have been sufficiently small to be reflected in the significant manner observed. We

TABLE 4. Family size distributions in five year-classes of wire-tagged coho salmon. Regression equation for $V_{kf}:Y$ = 3.99 (mean) − 6.551 (calculated from year-classes 1967–77 where r = 0.946).

Family size	No. of families in year-class					Family size	No. of families in year-class				
	1967	1968	1971	1974	1977		1967	1968	1971	1974	1977
0			9	5	1	18	2				1
1			7	12	1	19	1				1
2			8	5	1	20					1
3		2	10	5	1	21		1			
4			4	2		22	2				
5	2	4	2	1		23	1				
6	1	3	2			24	1				
7	2	5			2	25					1
8	1	3	1	1		26	1				
9		2			2	27					
10	2	5	1		1	28	2				
11	1	2		1	2	29					
12	3	1	1		1	30					
13	5	1			2	31					
14		1			1	32					
15					1	33					
16	2				1	34					
17						35	1				

	Year-class				
	1967	1968	1971	1974	1977
Variance in family size	58.67	13.22	6.76	5.49	42.76
Mean family size	15.50	8.43	2.69	2.16	11.19
No. of tagged 2+ returns	465	253	121	69	235
No. of tagged families	30	30	45	32	21

TABLE 5. Correlation data on survival, effective size, and smolt (s) release number where $A = N_e$.

	B % s both sexes	C % s 2+ males	D % s 2+ females	E % s 1+ males	F No. of smolts released	G No. of 1+ males	H No. of 2+ males
A	0.744	0.753	0.734	0.535	0.329	0.501	0.759
B		0.995	0.998	0.781	0.172	0.613	0.811
C			0.987	0.764	0.151	0.589	0.798
D				0.786	0.184	0.625	0.816
E					0.427	0.920	0.867
F						0.691	0.628
G							0.899

$r_{AB\cdot E} = 0.618$, $r_{AB\cdot F} = 0.739$, $r_{AB\cdot G} = 0.667$, $r_{AH\cdot E} = 0.703$, $r_{AH\cdot G} = 0.815$, $r_{GH\cdot A} = 0.921$

Significance levels
Simple correlations: 0.05 = 0.514, 0.01 = 0.641
Partial correlations: 0.05 = 0.532, 0.01 = 0.661

may thus have seriously overestimated the actual number of males used: consequently, the true N_e may be sufficient to cause dangerous levels of genetic destabilization. It is of course possible, because there are no similar studies available for confirmation, that our effective numbers are indeed accurate and reflect the real effects of their magnitude. In effect, we are saying that dangerous levels of N_e cannot be stated with certainty, but they appear to have been reached at times in the Big Creek coho salmon population. We cannot rule out the possible importance of selection or genetic (heritable) influences on family size or age at maturity that may be operating during the time when effective numbers are small, thus contributing to the correlation observed with survival. Nei and Murata (1966) have shown, for example, that when fertility (the equivalent of family size) is inherited in a population that is stable in number, the effective size is expected to decline as heritability of family size (or fertility, or fitness) increases. The expectation that fitness is not highly heritable may not hold when environmental conditions vary between generations. When the further uncertainty about the true level of Wright's F is added, together with the fact that male parent number might be much less than estimated (the sperm from one large male can easily fertilize the eggs of 20 or more females), N_e may be about double the true N_e. These uncertainties could place numbers of each sex

TABLE 6. 95% confidence intervals (± parenthetical values) for percent expected 2+ survival (Y) at five levels of N_e.

	N_e				
	200	300	400	500	600
Y 22-year data set	0.60 (0.16)	0.83 (0.34)	1.06 (0.54)	1.29 (0.76)	1.52 (0.97)
Y 15-year data set	0.55 (0.19)	0.85 (0.44)	1.35 (0.74)	1.75 (1.05)	2.14 (1.37)

roughly in the range of 16−22 during less favorable years (numbers not wholly beyond believability) if the correlation between survival and N_e is to be trusted.

It is further conceivable that during years when parental N_e is small, there might be a tendency for more 1+ males to be produced, thus precluding their contribution to the numbers of 2+ males. If so, N_e and the 1+ return should be negatively correlated, a circumstance not supported by our results (Table 5).

Having scratched the surface of the effective size issue, it seems to be more important to explore the extent of the problem than to immediately find the true N_e. If our statement about the transfers of eyed eggs as the entire output of individual females is true a substantial part of the time, the problem we have perceived to exist in the hatchery is being exported to other stations as well. In fact, whenever transfers of salmonid eggs are made as described here, irrespective of the species involved, we envision the gradual decimation of genetic stability represented in the original gene pools, despite an apparently comfortable safety margin regarding the actual numbers of individuals in the brood stock.

Adequacy of the Models

Even though our calculations have been checked for accuracy, our assumptions, models, and the conclusions to which they lead may be questioned. First, we assumed that there was no generation overlap (1+ males strictly excluded from matings). Although this is a good approximation in the hatchery over the first 20 yr of data reported, we know that some 1+ males have been used in recent years. We also do not know the possible importance of 1+ contributions in natural matings downstream from the hatchery. Both 1+ contributions would be expected to increase N_e to a degree that we assume to be small, but concrete data are lacking.

Family Size Distributions

We noted earlier that family size distributions observed in the wire-tagged groups do not closely fit any simple distribution model. Although they can be "made to fit" Poisson, or rectangular distributions by choosing class-intervals "correctly," this contrivance is unnecessary for the present purposes and has been avoided. However, other aspects of family size must be considered more fully.

If the average number of progeny surviving from a round of matings is not two (e.g. Falconer 1967), the population must either be declining or expanding. However, in the coho data, family sizes much larger than two are common. The population is not expanding because most of the eggs (sometimes 95%) are transferred out of the system and, because adults tend to return to their place of release as smolts, they contribute no further to the numbers within the original donor hatchery. What seems

TABLE 7. Proportion of available adult females spawned for in-house smolt production.

Spawn year	Females available	Proportion spawned	Spawn year	Females available	Proportion spawned
1960	1679	0.291	1971	3429	0.087
1961	2426	0.216	1972	1724	0.204
1962	1377	0.585	1973	589	0.553
1963	3253	0.231	1974	906	0.396
1964	2658	0.213	1975	508	0.626
1965	2920	0.214	1976	1288	0.137
1966	7648	0.069	1977	270	0.811
1967	7064	0.041	1978	3207	0.074
1968	6441	0.042	1979	2320	0.088
1969	4720	0.074	1980	2855	0.101
1970	3913	0.079	1981	1979	0.198
Mean	4009	0.187	Mean	1734	0.298

TABLE 8. Comparison of mean family size in five year-classes of small parent numbers (marked) and larger parent numbers (unmarked).

Parent numbers	Year-class				
	1967	1968	1971	1974	1977
Small (marked)	15.50	8.43	2.69	2.16	11.19
Larger (unmarked)	36.73	30.27	7.12	1.33	33.17

then to be a very large capacity for increase (noting the average family size over 25 yr of about 12) is not realized, and in fact is contributing to the problem when the consequences of V_k/u_k on N_e are taken into account. Data in Table 4 suggest, somewhat ambivalently, that an increase in N_e would result in better survival; however, increased survival would be accomplished by reducing family size because a smaller proportion of fish saved from more numerous matings would be required, necessarily still remaining within the capacity limits of the hatchery. This reduction in family size leads to the (seemingly) contradictory situation of increasing survival by reducing fitness. Fitness of course is not really reduced, but simply exported; thus, an accurate fitness assessment would have to extend beyond the hatchery to include all recipient stations as well. A more direct fitness assessment would simply involve the measurement of changes in survival from smolt to 2+ within the hatchery by comparing groups of small and large effective size. Limited data are fortunately available from the wire-tagging study that may at least illustrate the point. The mean family sizes calculated for five year-classes of the unmarked hatchery returns (from Table 1) were compared with the family sizes of corresponding year-classes of wire-tagged fish that represented known (and smaller) numbers of matings. In general, the survival (mean family size) is substantially greater in the (much larger) unmarked groups (Table 8), averaging 8.0 for marked and 21.7 for unmarked. We thus have a second estimate of the consequences of effective size, again indicating that survival increases as N_e increases. A seemingly reasonable interpretation of the lesser family sizes in the tagged groups would be to explain them as the consequence of nonrepresentative sampling, in direct analogy to the case for random drift.

Strategies for Increasing N_e

A very appreciable fraction of the genetic potential (and

hence the genetic stability) of the Big Creek population may have been unintentionally discarded (Table 7). Several fairly simple strategies should suffice to increase the contribution of available adults to subsequent generations and thus increase N_e. Every effort should be made to obtain enough males to equal the number of females without recourse to 1+ males. Iwamoto et al. (1985) reported 4.6-fold increase in 1+ production when 1+ males are used. Such increases are at the expense of 2+ males, which are more valuable in the marketplace. The male deficit should be filled by bringing in males from nearby stations that have traditionally received Big Creek stock. Cooled sperm samples could also be imported. If importation of sperm or fish is restricted, consideration should be given to developing a cryopreservation program for later years. This action would also expand the genetic base of the population by introducing generation overlap. Eggs should be taken in one container per female. Sperm from one male is added to the first container, and that male is never used again; nor are subsequent males after a single use.

Single matings (as just described) are preferred to the use of pooled sperm or to the traditional procedure for two principal reasons. First, if the eggs of three of four females are combined and sperm from the same number of males is then added, there is risk that the contributions of the males might be unequal. Even if the sperm was well mixed prior to mixing with the eggs, motility differences among the sperm from different males could permit appreciable differences in contribution of the males. This factor should not be operative in single fertilizations, given that total sterility is probably uncommon. Similarly, the single matings avoid the problem of differences in volume of seminal fluid relative to volume of spermatozoa, which (to be accounted for accurately) would require measurement of packed cell volume and then adjustment of whole sperm accordingly. The whole idea of enabling equal contribution by males thus seems to be most simply served by using single matings without concern over sperm quantity or motility.

Second, eggs should be mixed before they are exported or used internally. This might be done in several ways, either when the eggs are recently water-hardened or at the eyed stage. A large container, at least twice the volume of the entire egg-take, could be used. Eggs could be mixed by using a stream of water. Subsequent egg distributions would represent maximum N_e and maximum use of the genetic potential of the population.

What is the Present Level of Risk in this Coho Salmon Stock?

Even if this judgment is based on the data in Table 7 alone, the risk to the stability of Big Creek coho salmon appears to be greater than prudent management would accept. Given that the correlation between survival and N_e was measured over a period of declining abundance, and not the reverse, there is no assurance that survival will respond to an increase in N_e in the manner we have suggested. This must be stated, because if genetic variability has been reduced to very low levels, numerical expansion alone may not be enough (see equation 1). Hybridization with other local stocks seems to be one recourse if the N_e strategy fails, although large differences between lines may provide a sufficient diversity increase when generations are overlapped. If survival does improve with increased N_e, this improvement may be taken as a suggestion that some degree of genetic variability remains. The lack of unequivocal information on how the population might respond to changes in N_e should not detract from the wisdom of increasing N_e promptly.

Returning to the matter of defining the extent of the effective size problem, we emphasize that it could be very much worse in the future if the breeding strategies of some extremists are taken seriously. Astonishingly, Billard (1985) stated in reference to trout breeding: "To summarize, a 1-kg male yields at least 200 billion spermatozoa; with a maximum of 200,000 spermatozoa per egg when inseminating, such a male can fertilize 1 million eggs, i.e. the eggs produced by 500 1-kg females. Such a ratio is applicable only in large hatcheries because a minimum number of males must be used to ensure sufficient genetic mixing. This technique is widely used in Japan (Nomura, personal communication)."

At the fecundity rate used by Billard, and if the size of Big Creek Hatchery were doubled (to accommodate 5 million eggs), this expansion would represent 2500 females and 5 males. Then N_e (from equation 6) would be about 20 (10 males and 10 females). This number suggests that problems would develop very soon, even if V_k/u_k and other factors influencing N_e were ignored.

The importance of these generalities extends beyond the requirement that stocks be maintained in the best possible state of genetic integrity. For example, if stock composition in mixed-stock fisheries is to be estimated by electrophoresis techniques (e.g. Fournier et al. 1984; Beacham et al. 1985), the presence of random drift from year to year in one or more of the stocks may seriously compromise such attempts if hatchery stocks are involved.

We emphasize that maximization of N_e dictates that more must be done than simply specifying that some minimal number of parents is required for brood stock purposes (Ryman and Ståhl 1980). The crux of the present paper is that the sufficiency of parent numbers depends on factors other than census numbers alone and might well be a unique feature of each stock that could fluctuate as environments change. Because of the paucity of information on these other factors in most stocks, the temptation to specify what might be a "sufficient" minimal number of each sex should be approached cautiously (meaning generously). Even so, this should not detract from the desirability of increasing N_e in many populations.

In conclusion, one might examine more fully Brown's (1982) contention that Pacific salmon hatcheries tend to compromise the natural stocks they propagate rather than perpetuating them as was their intended purpose. Brown quoted several respected fish experts in support of this contention and added a litany of examples from the Pacific Northwest in which mitigation hatcheries have failed to meet the salmonid production levels once supported by habitats that are now altered or destroyed. In the context of the present paper, one might suppose that hatcheries per se may not be entirely at fault and that they could conceivably do much better if their managers were instructed to give every possible mating a chance to be represented at the smolt stage, a prominent feature in natural matings.

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Use of Hatchery Coho Salmon (*Oncorhynchus kisutch*) Presmolts to Rebuild Wild Populations in Oregon Coastal Streams

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We evaluated the effectiveness of using hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. Juvenile and adult populations were monitored in 15 stocked and 15 unstocked streams from summer 1980 until summer 1985. During the summers following the planting of presmolts, the number of juveniles per square metre of pool surface area was higher in the stocked streams than in the unstocked streams. However, wild juveniles were significantly less abundant in the stocked streams during the 2 yr when density of wild juveniles was estimated separately from hatchery juveniles. Adult returns to the stocked streams were not significantly different from adult returns to the unstocked streams, but returns tended to be earlier in the stocked streams than in the unstocked streams. Despite similar numbers of adults per kilometre in the stocked streams and unstocked streams in the years the presmolts returned to spawn, the resulting densities of juveniles in the stocked streams were significantly lower than the densities of juveniles in the unstocked streams. We concluded that the early time of spawning of the hatchery coho salmon was largely responsible for their failure to rebuild the populations in the streams stocked with presmolts.

Les auteurs ont évalué l'efficacité de l'utilisation de pré-smolts de saumon coho (*Oncorhynchus kisutch*) d'élevage pour le rétablissement des populations sauvages de cours d'eau côtiers de l'Oregon. Des populations de juvéniles et d'adultes de 15 cours d'eau empoissonnés et de 15 cours d'eau non empoissonnés ont été contrôlées de l'été 1980 à l'été 1985. Au cours des étés suivant le peuplement par pré-smolts, la concentration de juvéniles par mètre carré de surface de fosse était plus élevée dans les cours d'eau empoissonnés. Les juvéniles sauvages étaient cependant significativement moins abondants dans les cours d'eau empoissonnés au cours des 2 ans où la densité des juvéniles sauvages a été estimée séparément de celle des juvéniles d'élevage. Les remontées d'adultes vers les cours d'eau empoissonnés ne différaient pas de façon appréciable de celles des autres cours d'eau, mais avaient tendance à être plus hâtives. En dépit de concentrations similaires d'adultes (nombre par kilometre) dans les deux types de cours d'eau les années où les pré-smolts retournaient frayer, les densités de juvéniles dans les cours d'eau empoissonnés étaient significativement inférieures à celles des cours d'eau non empoissonnés. Les auteurs concluent que la fraie hâtive des saumons cohos d'élevage explique en grande partie leur incapacité à rétablir les populations dans les cours d'eau empoissonnés de pré-smolts.

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The abundance of wild coho salmon (*Oncorhynchus kisutch*) in Oregon coastal streams has declined greatly since the mid-1960s (Oregon Department of Fish and Wildlife (ODFW) 1982). As a result, ocean fisheries for coho salmon have been severely restricted (Pacific Fisheries Management Council (PFMC) 1984). One by-product of the restricted fisheries has been the return of more adults to Oregon hatcheries than are needed to fill quotas for smolt production.

In response to the problem of abundant hatchery and scarce wild coho salmon, the ODFW began a restoration program to rebuild populations of wild coho salmon. The release of offspring of surplus hatchery spawners into coastal streams as unfed fry or as fed fingerlings (presmolts) has been a major component of this program. During the first 3 yr of the program (1980–82), approximately 3.4 million unfed fry and 14 million presmolts were released into coastal streams. The Alsea River, Siletz River, and Trask River stocks of hatchery coho were used in the program. Because they have been in the hatcheries for many generations, these stocks typically spawn much earlier than wild coho in Oregon coastal rivers.

Although hatchery steelhead (*Salmo gairdneri*) may not survive as well in the wild as wild steelhead (Reisenbichler and McIntyre 1977; Chilcote et al. 1984), similar data are not available for coho salmon. In our study, presmolt hatchery coho salmon were released in an attempt to rebuild populations of wild coho salmon. The objectives were to determine if the release of hatchery presmolts resulted in (1) higher densities of juveniles in the stocked streams in the year of release, (2) more spawners in the stocked streams upon their return 3 yr hence, (3) higher densities of juveniles in the next generation, and (4) undesirable effects on populations of wild coho salmon.

Methods

Study Streams

Fifteen study streams were selected from the approximately 100 coastal streams that were stocked with hatchery coho pre-

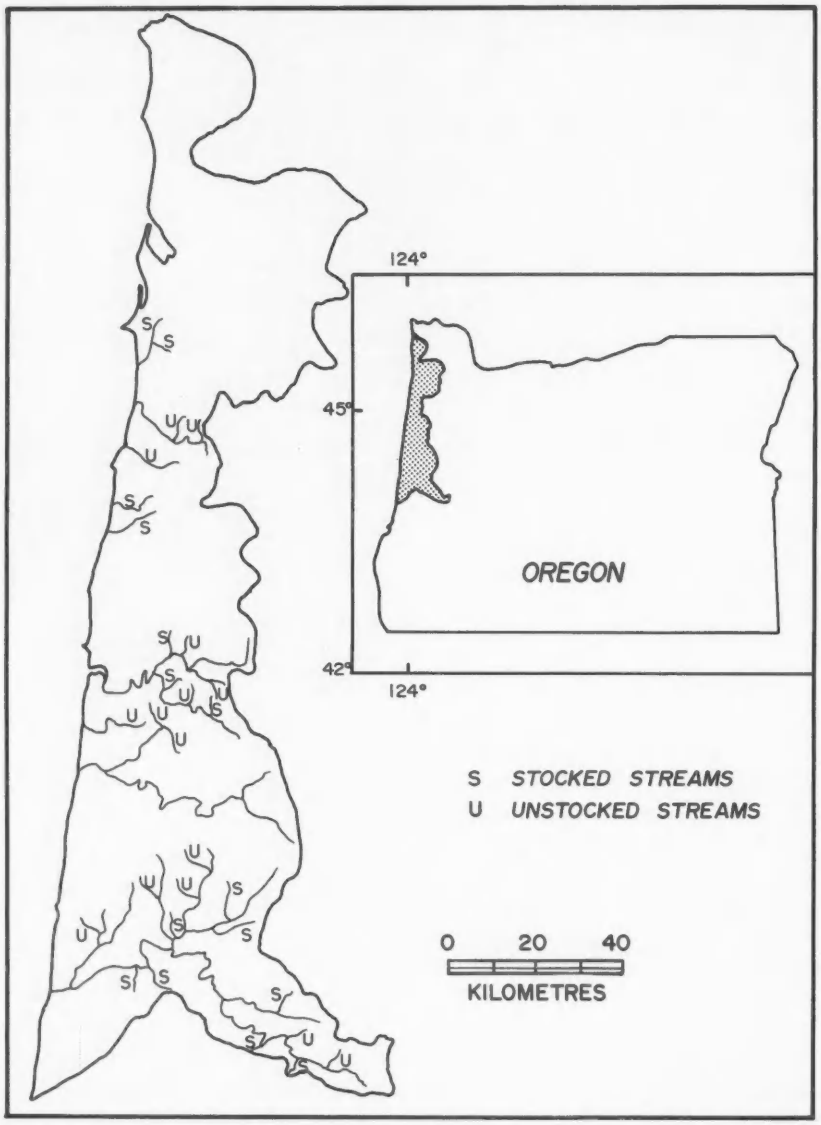

FIG. 1. Location of the presmolt evaluation study streams relative to the geographical distribution of the coho presmolt releases (shaded area).

smolts in 1980. An additional 15 unstocked streams were selected as controls. The 30 streams were distributed over nine Oregon coastal basins (Fig. 1).

The hatchery presmolts were planted during April and May of 1980–82. Presmolts averaged 2.25 g (62 mm) and were stocked in the accessible portion of each stream at an average density of $3.9/m^2$ of expected summer surface area. Known numbers of presmolts marked with a clipped adipose fin and a coded-wire-tag were released in 11 streams in 1981 and in 15 streams in 1982. The populations in the stocked and unstocked streams were sampled each summer from 1980 to 1985 as juveniles (6 yr) and each fall and winter from 1980–81 to 1984–85 as adult spawners (5 yr).

Adult Density

The evaluation was designed to determine whether stocking resulted in a change in the average spawner density in run years 1982–84 from the average spawner density in run years 1980–81 (Table 1). Run years begin in October of the year indicated and end the following March. Spawner density in the unstocked streams was monitored to account for trends that were unrelated to the stocking, such as changing harvest rates.

Spawning surveys of live adult coho salmon were conducted by walking index reaches of each study stream approximately weekly from mid-October until mid-February (except in 1984–85 when low flows extended the spawning season to mid-March). Surveys were only conducted under conditions of

TABLE 1. Summary of the hypotheses. The data used to test each hypothesis are identified by number (H_1—H_4). Population components are listed as wild (W), hatchery (H), or hatchery offspring (HO).

Hypotheses

H_1: no difference in the average density of juveniles between the stocked and unstocked streams during the summers following stocking

H_2: no difference in the average density of wild juveniles between the stocked and unstocked streams following release of the presmolts

H_3: no differences between the average numbers of early, late, and total spawners between the stocked and unstocked streams during the years that the hatchery fish returned

H_4: no difference in the average density of juveniles between the stocked and unstocked streams during the summers following return of the hatchery fish

Adult run year	Population components: Stocked streams	Population components: Unstocked streams	Summer of juvenile sampling	Population components: Stocked streams	Population components: Unstocked streams
1979	W	W	1980	H + W	W
1980	W	W	1981	H + W	W
1981	W	W	1982	H + W	W
1982	H + W	W	1983	HO + W	W
1983	H + W	W	1984	HO + W	W
1984	H + W	W	1985	HO + W	W

good water clarity. This method has been shown to account for an average of 75.5 ± 1.4% of the adult coho present in index reaches of Oregon coastal streams (Solazzi 1984). Index reaches totaled 25.1 km in the stocked streams and 25.0 km in the unstocked streams. We made an area-under-the-curve (AUC) estimate of spawners (Beidler and Nickelson 1980; Neilson and Geen 1981) for each study stream. By this method, total spawners in an index reach were estimated by dividing AUC-estimated fish-days by an average residence time of 11.3 d/fish (the average of the values reported by Willis (1954) and Beidler and Nickelson (1980)). The estimated number of spawners was then divided by the length of stream surveyed to express density as spawners per kilometre.

We tested the hypothesis of no difference in average spawner density between the stocked streams and the unstocked streams for run years 1982–84 (poststocking period) by using analysis of covariance, with average spawner density in each stream in run years 1980–81 (prestocking period) as the covariate. This allowed us to account for any differences between the stocked and unstocked streams that existed prior to stocking (Anderson et al. 1980). We believe that the assumption that the covariable be measured without error is met because of the small amount of error in our estimates. We tested the same hypothesis for early spawners and for late spawners (fish counted before December 1 and after November 30, respectively). December 1 was chosen because in almost all years, spawning at Fall Creek Hatchery (Alsea River) is completed before this date (ODFW, unpubl. data) and thus the vast majority of fish counted in the stocked streams after December 1 would be of wild origin.

Juvenile Density

The evaluation was designed to determine if juvenile densities changed because of the release of hatchery presmolts and to determine if any differences were retained in the next generation. We tested the hypotheses that there was no difference in juvenile density between the stocked and unstocked streams in each period (1980–82 and 1983–85) (Table 1).

In each stream, 15 study reaches were chosen by selecting three units of five consecutive pools. In the stocked streams, the units were located at points 20, 50, and 80% of the distance from the mouth to the uppermost stocking site. In the unstocked streams, the units were located at points 20, 50, and 80% of the distance from the mouth to the upper limits of coho distribution. The surface area of each pool and the riffle immediately upstream were measured and the juvenile population was estimated by the removal method (Seber and LeCren 1967). Populations consisted almost exclusively of age 0 coho because coho in Oregon typically smolt after 1 yr in freshwater. All population estimates and all pool surface areas were summed for each stream and the population density for each stream was expressed as fish per square metre of pool because coho were almost never found in the riffles.

We tested the hypotheses of no difference in average density of juvenile coho salmon between stocked and unstocked streams for the years 1980–82 (presmolt stocking years) using

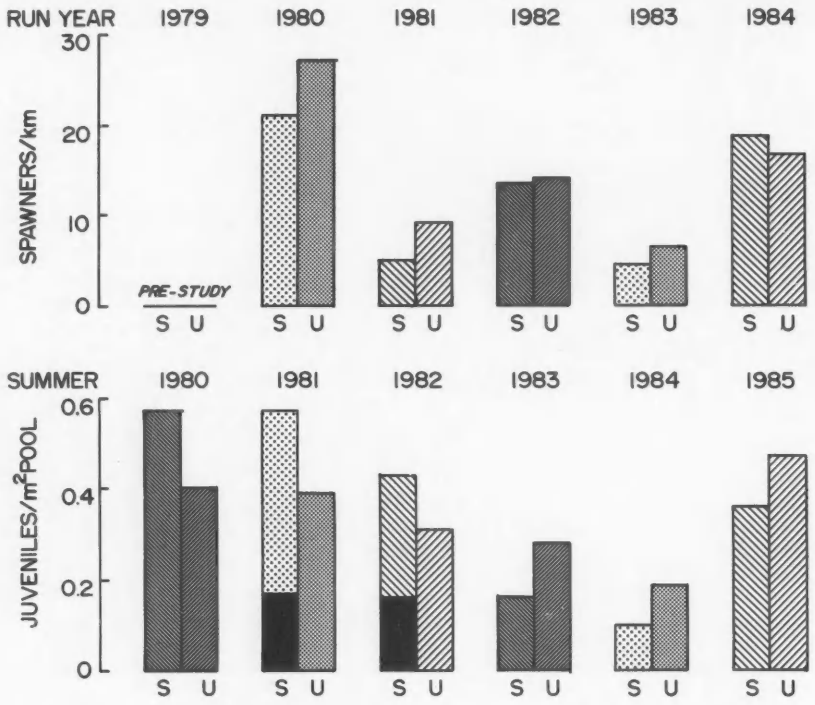

FIG. 2. Average density of adult and juvenile coho salmon in stocked (S) and unstocked (U) streams. Streams were stocked with hatchery presmolts in springs 1980–82. Similar shading represents the brood cycle. Solid portions of juvenile bars for stocked streams in 1981 and 1982 designate wild juveniles.

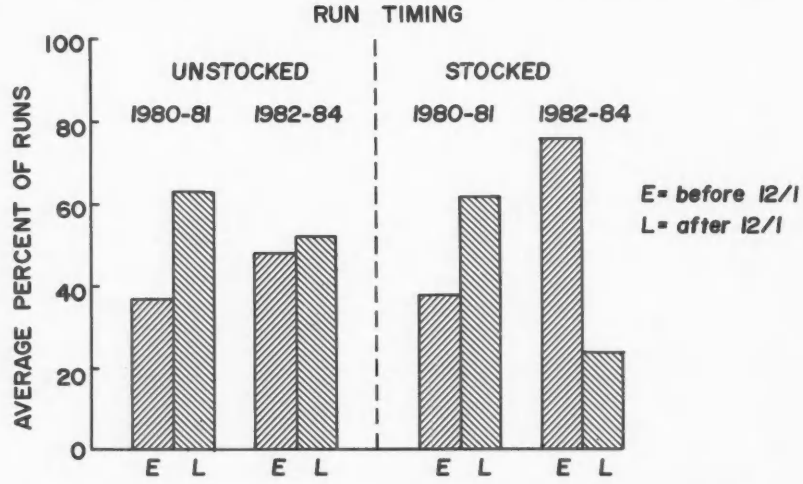

FIG. 3. Comparison of the proportion of early (E) and late (L) spawners (before and after December 1) in the stocked and unstocked streams for 1980 and 1981 (the prestocking period) and for 1982–84 (the poststocking period).

a two-tailed *t*-test. We used analysis of covariance, with spawner density as the covariate, to test the same hypothesis for the years 1983–85 (presmolt offspring years). This allowed us to account for any differences that might exist in spawner density between the stocked and unstocked streams.

Interaction Between Hatchery and Wild Juveniles

We had no direct measure of the density of wild juveniles in the stocked streams prior to stocking because the hatchery presmolts were stocked at a time when wild juveniles were still emerging from the gravel. Therefore, we used an indirect ap-

TABLE 2. Average densities of juvenile coho salmon (fish/m^2 of pool) in 15 stocked and 15 unstocked streams, 1980–85. Standard deviations are in parentheses. Adjusted values are adjusted for differences in spawner density between streams.

Group	Treatment		
	Stocked	Unstocked	*P*
1980–82 total juveniles	0.52 (0.21)	0.37 (0.18)	0.036
1981–82 wild juveniles	0.18 (0.14)	0.35 (0.16)	
Adjusted	0.19	0.34	0.010
1983–85 wild juveniles	0.21 (0.11)	0.31 (0.17)	
Adjusted	0.21	0.31	0.045

proach to determine the impacts of planting hatchery presmolts on the wild populations. Because the study streams were chosen randomly and the stocked and unstocked streams were geographically interspersed (Fig. 1), we assumed that the number of juveniles produced per spawner was similar for the stocked and unstocked streams. Then, for a given number of wild spawners, we would expect to find the same average density of wild juveniles in the stocked streams as we found in the unstocked streams if no effects of hatchery presmolts on wild juveniles had occurred.

We estimated the number of wild juveniles in stocked streams containing marked presmolts by subtracting the estimated number of hatchery coho salmon in each reach from the population estimate for that reach. The number of hatchery coho salmon in each reach was estimated (H) using the following equation:

$$H = (n/p)(N/c)$$

where n = the number of marked coho salmon captured, p = the proportion of marked coho salmon stocked, N = the total population estimate, and c = the total number of coho salmon captured (marked and unmarked). Estimates of the wild populations were summed and the density of wild juveniles for each stream was expressed as fish per square metre of pool.

We tested the hypothesis that there was no difference in average density of wild juveniles between the stocked and unstocked streams in 1981 and 1982 (Table 1) by using analysis of covariance, with spawner density as the covariate. This allowed us to account for any differences that might exist in spawner density between the stocked and unstocked streams.

Results

During the summers following planting of hatchery coho salmon presmolts (1980–82), average density of all juveniles in the stocked streams was 41% higher ($P = 0.018$) than the average density of juveniles in the unstocked streams (Fig. 2; Table 2). However, after adjusting for differences in spawner density, the average density of juvenile wild coho salmon in the stocked streams in 1981 and 1982 was 44% lower ($P = 0.010$) than the average density of juvenile wild coho salmon in the unstocked streams in 1981 and 1982 (Table 2).

After adjusting for differences in adult density among streams in the prestocking period, there was no difference ($P = 0.642$) in average density of spawners between the stocked and unstocked streams in the years the presmolts returned as adults (Fig. 2; Table 3). Whereas spawner density in the poststocking period was not different between the stocked and unstocked streams, there was a large shift from late spawners to early spawners in the stocked streams (Fig. 3). After adjusting for differences in density of early and late spawners among streams in the prestocking period, the average densities of early and late spawners in the stocked streams during the poststocking period were 70% higher ($P = 0.73$) and 48% lower ($P = 0.035$), respectively, than the average densities of early and late spawners in the unstocked streams (Table 3).

TABLE 3. Average density (adults/km) of early (before December 1), late (after November 30), and total coho spawners in 15 stocked and 15 unstocked streams. Standard deviation is in parentheses. Adjusted values are adjusted for prestocking differences in spawning density between streams.

Period	Treatment		
	Stocked	Unstocked	*P*
Prestocking (1980–81)			
Early	4.8 (6.7)	6.5 (7.9)	
Late	8.0 (7.6)	11.3 (9.5)	
Total	12.8 (13.7)	17.8 (16.5)	
Poststocking (1982–84)			
Early	9.4 (7.6)	6.0 (4.7)	
Adjusted	9.7	5.6	0.072
Late	3.0 (1.9)	6.4 (4.8)	
Adjusted	3.2	6.1	0.035
Total	12.3 (8.2)	12.3 (8.1)	
Adjusted	13.0	11.7	0.642

Although total spawner density in the 1982–84 runs (the years that the presmolts returned) was not different between the stocked and unstocked streams, density of juveniles in the stocked streams the following summer averaged 32% lower ($P = 0.045$) than the density of juveniles in the unstocked streams (Fig. 2; Table 2).

Discussion

The release of hatchery coho salmon presmolts during late spring increased by 41% the overall density of juveniles rearing in the stocked streams during the summer following release. In six tributaries stocked with hatchery coho presmolts in the Clearwater River basin of Washington, the summer densities of juveniles were 200–300% higher than the summer densities of juveniles in unstocked tributaries during the preceding 2 yr (S. B. Mathews, University of Washington, Seattle, WA, pers. comm.). However, in our study, 44% of the wild juveniles in the stocked streams were replaced by hatchery presmolts. We believe this replacement was the result of competition between smaller wild coho and the larger hatchery coho. The hatchery presmolts were released at an average length of about 62 mm at a time when newly emerged wild juveniles would have been about 39 mm in length (Moring and Lantz 1975). This size advantage for the hatchery presmolts was due to an earlier spawning time and to 2 or 3 mo of feeding in the hatchery. Chapman (1962) and Mason (1966) found that for wild coho salmon, larger individuals dominated smaller individuals causing their emigration or death.

On the average, density of spawners in the stocked streams was not statistically different as a result of the release of hatchery presmolts. Whereas a few individual streams did experience substantial increases, most did not. Because of the large amount of variation in spawning density among streams, the average for the stocked streams would have had to have been 56% greater than the average for the unstocked streams for us

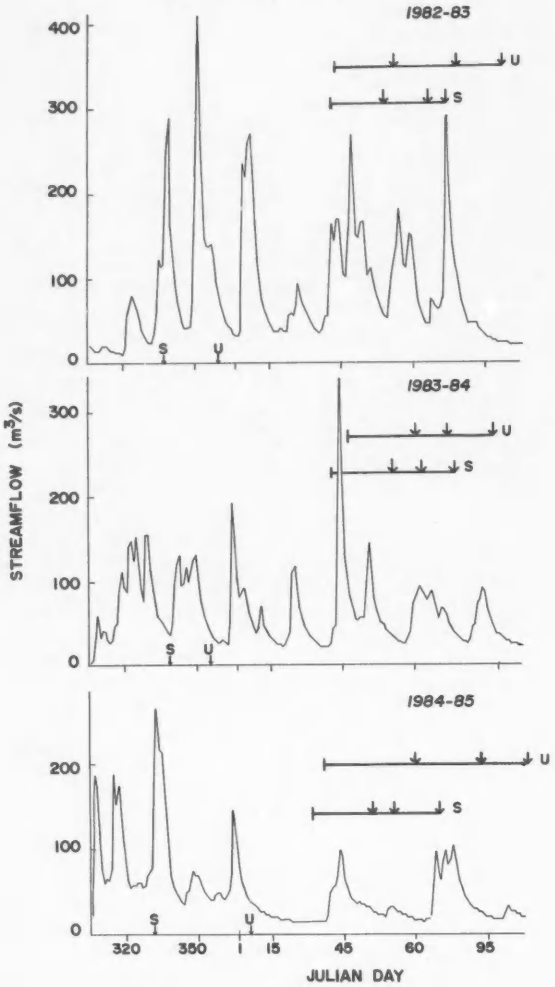

FIG. 4. Average daily streamflow for the Nestucca, Siletz, Yaquina, Alsea, and Siuslaw river basins, November through April. The arrows on the horizontal axis designate the time at which 75% of the spawners had been observed in the stocked (S) and in the unstocked (U) study streams. The estimated period from beginning until 75% emergence is shown by the horizontal lines labeled S and U. The arrows represent the completion of 25, 50 and 75% of emergence.

to detect a difference at the 90% level of significance, 80% of the time. The average density of spawners in the unstocked streams declined during the poststocking period compared with the prestocking period because of reduced survival and increased rate of harvest of adults returning in run year 1983 (Fig. 2), the result of El Niño (Johnson 1984). The average density in the stocked streams during the poststocking period was about the same as during the prestocking period despite the poor returns in 1983. This result suggests that the stocking of presmolts probably had a net positive effect on number of spawners, but that our tests were not sensitive enough to detect it statistically.

The density of late spawners in the stocked streams during the poststocking period was 48% less than the density of late spawners in the unstocked streams, probably the result of the decrease in wild juveniles of the same broods in the stocked streams. When combined with a 70% greater occurrence of early spawners in the stocked streams due to the return of hatchery adults, this resulted in the average time of spawning in the stocked streams during the poststocking period being about 2.5 wk earlier than the average time of spawning in the unstocked streams.

We believe that the reduced density of juvenile coho salmon in the stocked streams compared with the density of juvenile coho salmon in the unstocked streams during the summers following the 1982–84 spawning runs was related to the shift in the time of spawning in the stocked streams. We believe that the early spawners contributed very little to juvenile recruitment because juvenile abundance in the 30 study streams during 1983–85 was correlated with density of late spawners ($P < 0.001$) but not with density of total spawners ($P = 0.350$). Streamflow conditions during the periods following spawning and following emergence could explain the poor contribution of the early spawners. The shift toward an earlier time of spawning and the reduction in absolute numbers of late spawners translated into an earlier period of emergence for the stocked streams compared with the unstocked streams. Hartman et al. (1982) speculated that earlier emergence of coho salmon fry in Carnation Creek, British Columbia, resulted in greater emigration of the fry because of the increased occurrence of freshets early in the year. Cramer et al. (1985) found a negative relationship between survival of spring chinook (*Oncorhynchus tshawytscha*) from egg to fry and winter flows in the Rogue River, Oregon.

An examination of the flow records for five of the coastal basins where our study streams were located (Hubbard et al. 1984; U.S. Geological Survey, unpubl. data) showed that for run years 1982–84, 18 freshets occurred following peak spawning in the stocked streams and 13 freshets occurred following peak spawning in the unstocked streams (Fig. 4). We estimated emergence timing by assuming an average 108 d (Moring and Lantz 1975) from adult observation until emergence. We found that four freshets occurred following 50% emergence in the stocked streams compared with only one freshet following 50% emergence in the unstocked streams.

The importance of freshets in reducing survival of early spawned coho salmon appears to be demonstrated by the low flows experienced by coho salmon spawned in run year 1984. Fewer and smaller freshets occurred in 1984–85 compared with 1982–83 and 1983–84 (Fig. 4). The average density of juveniles in the stocked streams in 1985 was 23% less than the average density of juveniles in the unstocked streams. However, the average densities of juveniles in the stocked streams in 1983 were 43% less and in 1984 were 47% less than the average densities of juveniles in the unstocked streams.

We conclude from this study that hatchery coho salmon should have the same time of spawning as the wild coho salmon they are supplementing and they should be similar to the wild fish in size at the time of release. We are currently beginning an evaluation of the use of a broodstock that meets these guidelines.

As a result of our study, the release of hatchery presmolts in Oregon coastal streams is currently being concentrated in areas without wild populations. In addition, attempts are being made to identify and develop broodstocks that would be more appropriate than present hatchery stocks for supplementing populations of wild coho salmon.

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Conductivity–Temperature Standardization and Dissolved Solids Estimation in a Meromictic Saline Lake

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Hall, K. J., and T. G. Northcote. 1986. Conductivity–temperature standardization and dissolved solids estimation in a meromictic saline lake. Can. J. Fish. Aquat. Sci. 43: 2450–2454.

Studies on seasonal changes in micro-stratification in meromictic Mahoney Lake required investigation of appropriate temperature–conductivity (T–C) adjustments and conductivity – dissolved solids relationships. In situ conductivity measurements were adjusted to 25°C using a series of T–C relationships determined in the laboratory for Mahoney Lake water collected from different depths and at different times. Comparisons were made between conductivity determined from in situ measurements, conductivity in diluted water, and chemical analysis. Conductivity measurements were evaluated as estimates of the dissolved solids content of the water. T–C relationships were found to change considerably with depth and season in salt-stratified lakes, and errors as high as 15% could occur in Mahoney Lake unless several T–C plots were used to adjust field conductivity measurements to a constant temperature. Corrected in situ measurements underestimated conductivity as determined from the chemical composition. Diluted conductivity provided a more consistent estimate of dissolved solids content by eliminating most effects of ion–ion interactions upon ion mobility.

L'étude des variations saisonnières de la micro-stratification dans le lac Mahoney, un lac méromictique, nécessite d'apporter certaines corrections température–conductivité (T–C) et de tenir compte des relations entre la conductivité et les matières dissoutes. Des mesures in situ de la conductivité ont été corrigées pour une température de 25°C à l'aide d'une série de relations T–C déterminée au laboratoire à partir d'échantillons d'eau du lac Mahoney prélevés à des profondeurs et à des moments différents. Des comparaisons ont été faites entre les valeurs de conductivité déterminées lors des mesures in situ, celles déterminées en eau diluée et celles de l'analyse chimique. Les valeurs de conductivité ont été évaluées en tant qu'estimations de la teneur en matières dissoutes de l'eau. Les relations T–C variaient de façon importante en fonction de la profondeur et de la saison dans les lacs à stratification saline et, pour les eaux du lac Mahoney, des erreurs pouvant atteindre 15 % étaient obtenues si l'on n'utilisait pas plusieurs courbes T–C pour corriger en fonction d'une température constante les valeurs de conductivité déterminées sur place. Les valeurs de conductivité corrigées obtenues in situ étaient inférieures à celles déterminées par analyse chimique. Les valeurs mesurées à partir d'échantillons dilués permettaient d'obtenir une estimation plus cohérente de la teneur en matières dissoutes par élimination de la plupart des effets des interactions ion–ion sur la mobilité ionique.

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Conductivity is a measure of ion mobility in an aqueous solution. The mobility of ions changes as a function of temperature. Therefore, any study in a lake to examine vertical, seasonal, and yearly changes in dissolved ions by conductivity measurements requires that a temperature adjustment be made. In standard practice, temperatures are usually adjusted to 18 or 25°C (Edmondson 1956; American Public Health Association (APHA) et al. 1985), and conductance has been found to increase by 1.9%·°C^{-1} (APHA et al. 1985) or as much as 2.5%·°C^{-1} (Smith 1962). In a more detailed investigation of temperature–conductivity (T–C) relationships, Smith (1962) found that the temperature coefficient could vary for different lakes and he also showed seasonal changes. He recommended that temperature coefficients be determined for each water body studied. In meromictic and other lakes where ionic contributions change markedly with depth, a carefully selected series of temperature coefficients should be utilized.

Conductivity measurements have been used for many years to estimate the dissolved solid content of lakes (Edmondson 1956; Walker 1973; Brock and Shiel 1983). The relationship between dissolved ions and conductivity should be linear for simple dilute solutions, but as the concentration changes or the proportion of solutes change, the slope of this relationship also should change (Hem 1959). In spite of this, several investigators have used a simple conductivity – total dissolved solids (TDS) relationship to make comparative studies of saline lakes and to examine vertical and seasonal changes. Williams (1966) observed a linear relationship between conductivity and TDS in 41 Australian lakes dominated by NaCl and having TDS values from 0.5 to >200 g·L^{-1}. The conductivity–TDS relationship also has been used to investigate stability and sea-

sonal changes in several meromictic lakes in central Washington (Walker 1974). Hammer et al. (1978) have used vertical temperature and conductivity measurements to study seasonal and yearly changes in stratification and mixing in meromictic Waldsea Lake located in Saskatchewan.

In a comparative investigation of two saline lakes in the south Okanagan Valley of British Columbia, Northcote and Hall (1983) showed that an upper zone of sharp stratification occurred well above the permanent primary chemocline in meromictic Mahoney Lake in early spring. This secondary chemocline exhibited seasonal changes that could be important in regulating nutrient cycling and zonation of zooplankton populations. To examine the seasonal changes and stability of this micro-stratification, we had to make appropriate conductivity adjustments to a standard temperature and develop a detailed understanding of the conductivity–TDS relationship in the lake. This study reports on the techniques used in these adjustments and their relationships.

Materials and Methods

Water samples were collected from Mahoney Lake (see Northcote and Hall (1983) for detailed location and morphometric characteristics) with an electric bilge pump using a weighted 24 mm ID clear plastic hose with a horizontal four-directional sucking head. The samples were transported in a cooler and stored at 4°C in the laboratory for a period of 2–3 mo until all analyses were made.

Vertical temperature and conductivity measurements at a central station in the lake were made with a YSI model 33 T–C meter. Laboratory conductivity measurements were made with a Radiometer conductivity meter (CDM3) fitted with a Radiometer CDC 304 conductivity probe. Both meters were calibrated with 0.01 *M* KCl and the probes were cleaned after each immersion in the H_2S-rich monimolimnion of Mahoney Lake with an acidic isopropanol solution (10 parts distilled water to 10 parts isopropanol to 1 part concentrated HCl).

Water samples used to establish the T–C relationships were cooled in 200-mL beakers to 0–1°C in a freezer and the conductivity and temperature (calibrating mercury thermometer, Fisher No. 822–793) were measured at 1°C increments as the stirred sample warmed to room temperature. Heat applied to the outside of the beakers from our hands was used to raise sample temperature above the ambient air temperature (21–23°C). Diluted conductivity measurements were made as outlined in APHA et al. (1985).

In situ conductivity measurements were adjusted to 25°C using the series of T–C relationships determined in the laboratory for Mahoney Lake water from different depths. Since T–C relationships were not determined for each depth at which an in situ observation was made, the field measurements were adjusted using the closest T–C plot that was available. The field conductivity measurements were adjusted to 25°C by calculation using the slope relationship or by graphical interpolation with calipers from the appropriate T–C plot.

To rationalize the use of the different T–C plots to adjust the vertical field conductivity measurements, the statistical difference ($\alpha = 0.05$) between the slopes of adjacent T–C plots and between T–C plots from similar depths at different sampling dates was determined. The regression comparison was derived from an algorithm presented by Zar (1984) and conducted on a DEC VAX-11/750, Berkeley UNIX computer.

TDS (filtrable residue) was determined by evaporation of membrane (0.45 μm) filtered samples to a constant weight at 104°C. Alkalinity was determined on 5- to 10-mL samples diluted to 100 mL with distilled water and titrated with 0.02 *N* H_2SO_4 to pH 8.3 and 4.5 using a pH meter (APHA et al. 1985). Sulphide samples were collected in 60-mL glass-stoppered bottles, preserved with 1 *N* zinc acetate and 6 *N* NaOH, and quantified by the iodometric titration procedure (APHA et al.

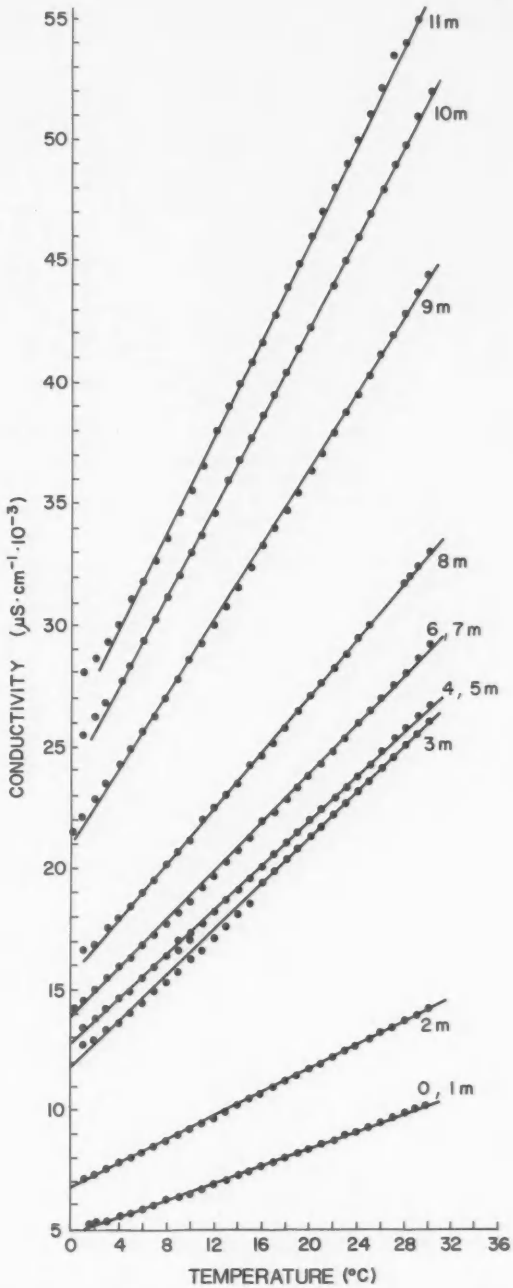

FIG. 1. Vertical changes in the T–C relationship for Mahoney Lake (8 December 1983).

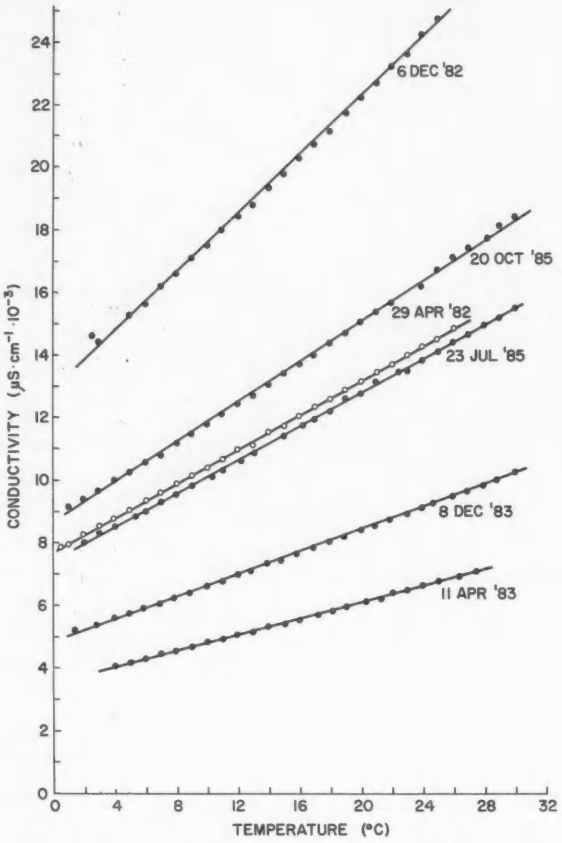

FIG. 2. Seasonal and yearly changes in the T−C relationship in the surface water of Mahoney Lake.

1985). It was necessary to correct the alkalinity titration for H_2S concentrations below the chemocline to calculate the HCO_3^- concentrations. Chloride was determined by the mercuric nitrate titration method and sulphate quantitated by the turbidimetric method (APHA et al. 1985). Calcium, magnesium, sodium, and potassium were determined by direct aspiration on an atomic absorption spectrophotometer (Jarrell Ash model 810) using an air/acetylene flame and a $CsNO_3$ ionization suppressant. Dissolved organic carbon was determined on acidified, filtered samples using a Beckman carbon analyzer (model 915). Volatile fatty acids (acetic, propionic, and butyric) were analyzed by injecting 1 μL of acidified (pH 2−3, 1% H_3PO_4) samples into a gas chromatograph (Hewlett Packard 5880A) operated under isothermal conditions (120°C) and equipped with a FID detector and a 1.8 m × 0.3 cm ID glass column packed with 0.3% Carbowax − 0.1% H_3PO_4 on 60/80 Carbopack C(Supelco, Inc.).

Results

Since Mahoney Lake shows considerable vertical variation in salinity (4−40‰), a vertical series of T−C plots was made from samples taken in December, to more accurately adjust all conductivity values to 25°C (Fig. 1). It was found that there was a gradual increase in the slope of the T−C relationship

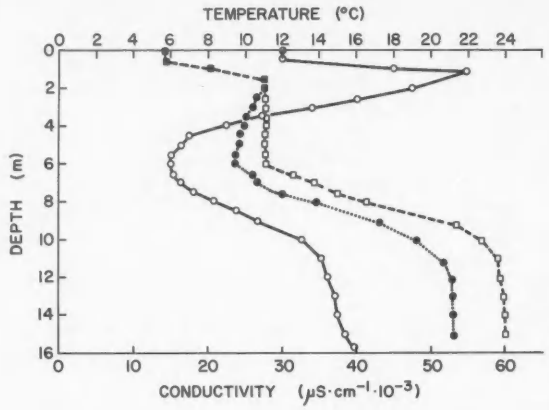

FIG. 3. Vertical temperature and conductivity in Mahoney Lake (29 April 1982). ○, temperature; ●, conductivity at 25°C corrected with single surface T−C adjustment factor only; □, conductivity at 25°C corrected with a series of vertical T−C adjustment factors.

with depth which was significant ($\alpha = 0.05$) except within the samples from 0−1, 3−4, 4−5, and 6−7 m. Thus, 8 of the 12 T−C plots made at 1-m intervals between 0 and 11 m were required to accurately correct the December 1983 field conductivity values to 25°C. The vertical changes in the in situ conductivity measurements could be used as a rough guide to select the depths at which samples should be taken to prepare the T−C plots.

There was also a significant difference among many slopes of the T−C plots made from surface samples collected at different times (Fig. 2). Thus, one set of T−C plots was not sufficient to make the necessary conductivity adjustments during a series of seasonal measurements. Furthermore, during collection, transportation, and laboratory manipulation, H_2S could be lost from water samples collected below the chemocline (deeper than 8.0 m) and any $CaCO_3$ precipitation would cause some reduction in conductivity compared with in situ measurements. For example, precipitation of $CaCO_3$ from the supersaturated conditions of 8 December 1983 to form a saturated solution would reduce the conductivity (25°C) by approximately 200 $\mu S \cdot cm^{-1}$. Corrections for such chemical changes require that T−C plots be made in the field at the time that samples are collected.

The difference between the graphs of the vertical conductivity profile of Mahoney Lake using a single T−C plot from surface water only and using both the surface water and a complete vertical series of T−C plots is not inconsequential. Figure 3 shows an appreciable increase in the conductivity of water (25°C) below 2 m, with values up to 15% higher in deeper waters, when a complete series of T−C plots is used to adjust the field conductivity profile.

Analysis for major cations and anions indicates that Mahoney Lake is mainly a sodium sulphate dominated lake (Table 1). However, a cation−anion balance indicated a deficiency of anions to achieve electrical neutrality (average $\Sigma^+/\Sigma^- = 1.14$). Either a major anionic element was not included or there were some analytical errors. The latter was unlikely at the magnitude of difference observed, but a small analytical error multiplied by a large dilution factor could contribute to this imbalance. Silicate was not included in these

TABLE 1. Chemical composition of Mahoney Lake (8 December 1983). All values in $mg \cdot L^{-1}$; 15.0-m values taken from 20 May 1983 data except HCO_3^- and CO_3^{2-} which were measured 4 May 1985. H_2S concentrations also determined on 4 May 1985 with pH's of 8.02, 7.58, and 7.30 used to compute HS^- contribution to Σ^- at the 9-, 10-, and 15-m depths, respectively. DOC = dissolved organic carbon.

Depth (m)	K^+	Na^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}	H_2S	DOC	Σ^+/Σ^-
0	260	1 920	33	640	5 000	699	638	46	0	140	1.07
0.5	268	1 900	34	650	5 000	369	647	45	0	145	1.14
1.0	242	1 840	33	680	4 400	377	644	46	0	147	1.25
1.5	252	1 960	33	700	5 000	381	634	48	0	147	1.18
2.0	304	2 460	31	800	6 200	458	732	66	0	154	1.16
2.5	632	4 800	25	1540	14 000	889	1 211	151	0	267	1.03
3.0	624	5 040	25	1550	13 200	916	1 220	157	0	279	1.12
3.5	720	5 760	23	1600	15 200	902	1 611	87	0	292	1.08
4.0	696	5 440	24	1620	15 200	938	1 457	78	0	279	1.05
4.5	704	5 600	24	1640	13 200	991	1 315	151	0	292	1.20
5.0	700	5 600	25	1640	14 000	998	1-327	186	0	292	1.14
6.0	690	5 600	30	1780	15 200	1070	1 451	199	0	298	1.08
7.0	745	5 950	30	1790	15 200	1070	1 446	209	0	315	1.13
8.0	800	7 300	33	2110	17 200	1220	1 539	199	0	326	1.20
9.0	1250	11 200	83	3280	22 800	1630	3 775	0	141	595	1.35
10.0	1490	13 399	95	4120	27 800	2220	5 036	0	466	795	1.31
15.0	1935	16 521	37	5380	46 580	3600	10 868	0	462	—	0.96

TABLE 2. Conversion factors for TDS calculations from conductivity measurements (vertical water samples, Mahoney Lake, 8 December 1983; conversion factors are values calculated from the TDS/conductivity ratio).

Depth (m)	TDS ($g \cdot L^{-1}$)	Diluted conductivity ($\mu S \cdot cm^{-1}$ at 25°C)	Conversion factor	In situ conductivity ($\mu S \cdot cm^{-1}$ at 25°C)	Conversion factor
0	9.6	16 900	0.56	10 500	0.91
1	9.6	17 400	0.55	10 370	0.92
2	12.1	22 600	0.54	12 900	0.94
3	25.3	44 600	0.57	23 900	1.05
4	27.1	50 100	0.54	24 700	1.09
5	27.6	49 600	0.56	24 600	1.12
6	29.9	58 100	0.52	26 100	1.14
7	30.3	52 600	0.57	26 500	1.14
8	36.4	65 600	0.55	30 600	1.18
9	52.6	93 700	0.56	36 750	1.43
10	65.3	114 100	0.57	45 500	1.43

calculations but its contribution was small (9 $mg \cdot L^{-1}$ in the mixolimnion and up to 82 $mg \cdot L^{-1}$ in the monimolimnion in May 1983) compared with other major components. Soluble reactive phosphorus was usually less than detection level (10 $\mu g\ P \cdot L^{-1}$). A possible source of anions to help achieve a charge balance could come from low molecular weight fatty acids, since dissolved organic carbon levels varied from 140 $mg \cdot L^{-1}$ in the surface waters to 795 $mg \cdot L^{-1}$ in bottom (15 m) waters. However, volatile fatty acid analysis indicated that acetic, propionic, and butyric acid were all $<1\ mg \cdot L^{-1}$.

In spite of this anomaly, the chemical composition was used to calculate the conductivity for comparison with in situ conductivity measurements. The in situ conductivity measurements, even when detailed temperature corrections were made, still underestimated the conductivity contributed by the individual ionic components (Fig. 4). For the example presented, in surface waters the calculated conductivity values were 72% higher and at depths of 10 m were 113% higher than the in situ specific conductivity measurements.

To avoid the effects of ion–ion interactions, it has been recommended that solutions be diluted to the range of 90–120 $\mu S \cdot cm^{-1}$ to appropriately account for the contribution of each separate ion to the total measured conductivity (APHA et al. 1985). For Mahoney Lake, the diluted conductivity values are very close to the calculated conductivity in its upper mixolimnion but are slightly higher (10–16%) in its monimolimnion (Fig. 4).

Conductivity can be used to provide a measure of TDS in order to consider the solute effects upon density and stratification of lake waters. The conductivity measurements are multiplied by a factor between 0.55 and 0.70 to convert conductivity to TDS as milligrams per litre (APHA et al. 1985). For saline waters this factor may be higher than 0.70. Factors were calculated for a vertical series of Mahoney Lake samples using both the diluted and in situ conductivity measurements (Table 2). With the laboratory diluted conductivity values a conversion factor of 0.55 ± 0.02 was calculated whereas the undiluted in situ specific conductivity values gave an average conversion factor of 1.12 ± 0.18. This latter set of conversion factors showed a gradual increase from surface waters (0.91) to the deeper water (10 m = 1.43) whereas the values determined using diluted conductivity showed very little variation.

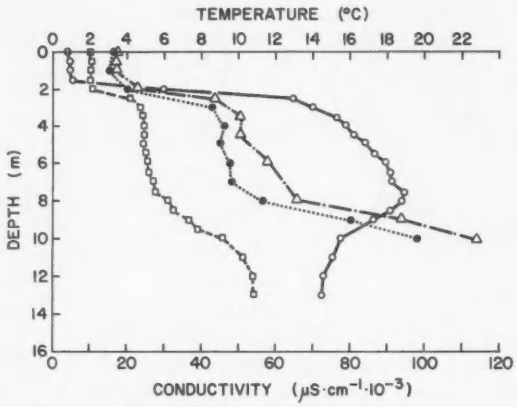

FIG. 4. Comparison of conductivity determinations in Mahoney Lake (8 December 1983). ○, temperature; □, in situ conductivity at 25°C corrected with a series of vertical T−C adjustment factors; ●, conductivity at 25°C calculated from ion concentrations, H_2S not included; △, conductivity calculated from diluted conductivity measurements at 25°C.

Discussion

Smith (1962) has shown that the T−C coefficient can vary for different waters and for seasonal changes in the chemical characteristics of the same water body. Our studies demonstrate that for salt-stratified lakes, the T−C relationship can change considerably with depth so that several T−C factors are necessary to accurately adjust field conductivity measurements to a constant temperature. Smith (1962) reported errors of 1% in adjusting conductivity measurements to 18°C using a single temperature coefficient for a particular water body. We would have had errors as high as 15% in the vertical conductivity profile of Mahoney Lake had we not also used the vertical series of T−C corrections. Temperature-compensated conductivity meters will only make a constant T−C slope correction, so they are inadequate when several T−C factors are required.

Both the concentration and the chemical composition of the electrolytes can affect the temperature coefficient. The vertical distribution of dissolved solids in Mahoney Lake changed by an order of magnitude (4−40‰ salinity). This concentration gradient causes changes in the ion−ion interactions which in turn affects ion mobility and response to temperature. There were also vertical and seasonal changes in the chemical composition of Mahoney Lake with variation in the molar ratios of some major cations and anions. The vertical change from oxic to anoxic conditions and the gradient of sulphide (up to 460 $mg \cdot L^{-1}$) and dissolved organic carbon (300−795 $mg \cdot L^{-1}$) in the monimolimnion would influence the solutes through precipitation and complexation reactions. Large differences in vertical temperature profiles (more than 20°C) and vertical pH changes (7.4−9.1) could affect the solution−precipitation equilibria of solids settling through the water column. These reactions could be further modified by seasonal weather patterns.

A difficulty in using in situ conductivity measurements as a measure of dissolved solids, even when appropriately adjusted for temperature, is that the equivalent conductivity decreases in concentrated solutions, such as those found in highly saline lakes, when compared with a high dilution. Many investigators attribute this decrease to ion pair and complex formation, although Horne (1969) attributed the retardation of ionic mobility in concentrated solutions to two drag phenomena, the relaxation and electrophoretic effects. Therefore, for greater accuracy, diluted conductivity measurements should be made. These can be related to TDS by multiplying by a constant conversion factor. If only temperature-adjusted in situ conductivity values are used to estimate dissolved solids in a salt-stratified lake, then the conversion factor will be larger and could vary with depth as well as seasonally, as occurred in Mahoney Lake.

Acknowledgements

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Adaptive Divergence of Trophic Phenotype among Freshwater Populations of the Threespine Stickleback (*Gasterosteus aculeatus*)

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Lavin, P. A., and J. D. McPhail. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). Can. J. Fish. Aquat. Sci. 43: 2455–2463.

We investigated differences in the morphological and behavioural contributions to foraging success among three morphotypes (open-water, littoral, and intermediate) of the threespine stickleback (*Gasterosteus aculeatus*) from a single drainage system on Vancouver Island. Stomach contents from wild samples showed diet to be dependent on morphotype. Interpopulation differences in trophic morphology were associated with differences in foraging success on given prey types. The longer jaw of the bottom-browsing littoral morphotype allowed it to ingest significantly larger prey then either the intermediate or open-water populations. In addition, the littoral type spent less time manipulating benthic prey then either the open-water or intermediate morphotype. The latter observation is independent of interpopulation differences in jaw length. Both the planktivorous open-water morphotype and intermediate morphotypes were superior foragers in experiments on water column prey. The differences in water column foraging success are associated with interpopulation differences in gill raker morphology. Our results support the conclusion that inter population divergence in trophic phenotype is an adaptive response to differences in the primary trophic resources available in lakes of different morphometry.

Nous avons étudié les différences dans le rôle joué par les aspects morphologiques et comportementaux du succès dans la recherche de nourriture entre trois types morphologiques (eau libre, zones littorale et intermédiaire) de l'épinoche à trois épines (*Gasterosteus aculeatus*) provenant d'un seul bassin hydrographique sur l'île Vancouver. Les contenus stomacaux provenant de spécimens sauvages ont montré que le régime alimentaire dépendait du type morphologique. Les différences entre les populations dans la morphologie trophique étaient associées aux différences observées dans le succès de recherche de nourriture sur des types de proie donnés. La mâchoire plus longue du type morphologique de la zone littorale qui broute au fond lui a permis d'ingérer des proies beaucoup plus grosses que les populations de la zone intermédiaire ou de la zone d'eau libre. En outre, le type de la zone littorale a consacré moins de temps à manipuler les proies benthiques que le morphotype d'eau libre ou de la zone intermédiaire. La dernière observation est indépendante des différences observées entre les populations dans la longueur de la mâchoire. Le morphotype d'eau libre planctonivore ainsi que les morphotypes de la zone intermédiaire étaient des organismes plus efficaces pour la recherche de nourriture dans des expériences sur les proies vivant dans la colonne d'eau. Les différences dans le succès de recherche de nourriture dans la colonne d'eau sont associées aux différences entre les populations dans la morphologie des branchiospines. Nos résultats appuient la conclusion voulant que la divergence entre les populations dans le phénotype trophique est une réaction d'adaptation aux différences dans les ressources trophiques primaires disponibles dans les lacs ayant une morphométrie différente.

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Reproduction isolation arising through adaptive divergence of subpopulations is thought to be a primary mode of speciation (review in Templeton 1981). Studies that focus on the historical patterns of population divergence typically attempt to correlate morphological, or genetic variability, with one or more environmental factors (e.g. Mitter and Futuyma 1979; Findley and Black 1983; Felley 1984); consequently, evolutionary histories are reconstructed primarily through inference (Mayr 1983). Such "adaptationist programs" have been criticized both for their inability to define the targets of natural selection and for their failure to consider other processes (Gould and Lewontin 1979). Inferential studies, however, can still correctly define selective processes, if the investigation includes a knowledge of both the organism's ecology (Clarke 1978) and a particular trait's functional significance (Bock 1980). Recently, this approach has allowed the direct measurement of natural selection in populations of Darwin's finches (Boag and Grant 1981). This study and others (e.g. Miles and Ricklefs 1984; Mittlebach 1984; Schluter and Grant 1984) concentrate on selection for divergence in trophic morphology (e.g. beak size) and its correlation with food type and availability.

In teleosts, modifications of trophic morphology are thought to be a common mechanism promoting such evolutionary phenomena as the explosive radiation of cichlids in the African Great Lakes (Liem 1973; review in Greenwood 1984). Almost all occurrences of these so-called "species flocks" involve some alteration of the feeding apparatus (review in Echelle and Kornfield 1984). Divergence in teleost trophic morphology is not limited to cases involving multiple radiations. In systems

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containing only a single species pair, interspecific differences in trophic morphology are correlated with resource partitioning (e.g. Lindsey 1981).

In the threespine stickleback (*Gasterosteus aculeatus*) species complex, interpopulation morphological variability is evidenced in many characters (review in Bell 1984). In at least one lake in British Columbia, biological speciation (sensu Mayr 1963) is associated with extreme divergence in trophic morphology, and this divergence has resulted in an almost complete separation of food types exploited by two species (McPhail 1984; Bentzen and McPhail 1984). In the present study we examine the significance of divergence in trophic morphology between different lacustrine populations of *Gasterosteus* found within a single drainage system. This system of lakes is in a recently deglaciated (approximately 10 000 yr B.P., Alley and Chatwin 1979) area and divergence is presumed to have proceeded from a single colonization event. Previously, Lavin and McPhail (1985) described interpopulation differences in trophic morphology for stickleback inhabiting different lakes in the Cowichan drainage system on Vancouver Island, British Columbia. Morphological differences were shown to be nonrandomly associated with lake type. Populations inhabiting small lakes dominated by benthic production were found to possess reduced gill raker length but increased upper jaw length relative to populations from large, open-water lakes.

Our purpose in the present study is to investigate the significance of these described differences with respect to feeding performance and diet. The hypothesis that interpopulation differences in trophic morphology constitute an adaptive divergence is supported (1) if interpopulation differences in trophic morphology are sufficient to produce detectable differences in foraging ability and (2) if the morphology associated with a particular resource (i.e. benthic or water column) increases the ability of individuals to utilize that resource.

Materials and Methods

Trophic Morphology

The trophic morphology of *Gasterosteus* in lakes in the Cowichan system (Vancouver Island) was detailed in Lavin and McPhail (1985). Three morphotypes were identified, each inhabiting one of three lake types: an open-water form associated with large lakes that are dominated by limnetic regions, a littoral form associated with much smaller lakes that are dominated by benthic regions, and an intermediate form from lakes of intermediate size, characterized by well-developed benthic and limnetic regions. In this study we examine the contributions of morphology and behaviour to foraging success. To this end we used a representative morphotype from each of the three lake types. The open-water morphotype was from a site (Caycuse) at the north end of Cowichan Lake, the littoral morphotype from a small bog lake (Grant Lake), and the intermediate morphotype from a small bay off the main body of Cowichan Lake (Bear Lake). Hereafter, these three populations are referred to as the "open water", "littoral", and "intermediate" morphotypes, respectively.

Three trophic variables were chosen for studies of functional significance: upper jaw length (length of the premaxilla), gill raker number, and gill raker length. Upper jaw length is thought to measure mouth gape, and consequently, in gape-limited predators this trait should be correlated with particle size (Aleev 1969). In fish, gill raker architecture (gill raker number, spacing, and length) is directly related to planktivory (Magnuson and Heitz 1971; Wright et al. 1983), and gill raker spacing is thought to regulate the size of the particle retained by planktivores (e.g. Kliewer 1970; Seghers 1975; Wright et al. 1983). For this reason we sought to identify interpopulation differences in gill raker spacing; however, because of the small size of laboratory-reared animals, precision was low for this measure. Consequently, gill raker density (GRD) was used as a surrogate measure of spacing. Gill raker number (GRN), the total number or rakers on the first gill arch, and gill raker length (GRL), the length of the longest raker on the first arch, were determined for individuals from each population. The arch was then excised from the opercular cavity and an enlarged tracing made using a Wild-M5 dissecting scope and camera lucida. Gill raker area (GRA), the total area occupied by the gill rakers, was determined by digitizing the tracings; Scheffe's test was then used to compare differences in relative area. Gill raker density was expressed as the number of rakers occupying 1 mm^2 (i.e. GRN/GRA). These proportional data were normalized using the arcsine square-root transform. The results were plotted against standard length, and differences in gill raker density among morphotypes were tested by analysis of covariance (ANCOVA).

We also attempted to generalize the response of both upper jaw length and gill raker number to trophic production among lacustrine populations of *Gasterosteus* in British Columbia. Mean gill raker number was obtained for 27 populations and mean upper jaw length measures for 19 populations. The influence of size on upper jaw length was removed within each population by adjusting upper jaw length to a standard length of 40 mm (Thorpe 1976; Lavin and McPhail 1985). Population mean upper jaw length was calculated from these adjusted data. Populations from small lakes dominated by benthic production are expected to have longer upper jaws, while open-water foragers from large lakes should possess numerous gill rakers.

A laboratory breeding program was conducted to assess the genetic contribution to character differences among morphotypes. Details of the methods and results from this breeding program are to be presented elsewhere; however, as this study is concerned with the adaptive divergence of trophic characters, some evidence that these characters are at least in part under genetic control is necessary. Thus, we present character means and standard deviations for wild and laboratory-reared samples of each morphotype. The retention of an interpopulation character difference among progeny raised under identical conditions implies a genetic contribution to the expression of that character (Mitchell-Olds 1986). Nine characters were scored for each morphotype: standard length, head length (HEAL), snout length (SNOL), eye diameter (EYED), upper jaw length (UPJL), gill raker number (GRN), head depth (HEAD), inner orbital width (INOW), and gill raker length (GRL) (Hubbs and Lagler 1958). These characters were size adjusted to a standard length of 40 mm. Differences between character means, among morphotypes, were tested by Scheffe's contrasts.

Morphotype Diet from Field Samples

The diet of each population was broadly characterized by examining gut contents of stickleback samples taken from each lake in late spring. The littoral morphotype is expected to have a diet dominated by benthic macroinvertebrates, while the

open-water population's diet should consist largely of water column plankton. Food items among individuals were pooled within each population and each item was classified as benthic or water column following Kliewer (1970). A test of nonradom association between food type and morphotype was made using a chi-square contingency test. In this instance, rejection of the null hypothesis would indicate that diet type, benthic or water column, is significantly contingent on morphotype. No individual within populations fed on a large number of atypical food items which might have biased the outcome of the test.

Although feeding patterns may change seasonally with dispersal to different lake areas after breeding, this should only accentuate dietary differences between the open-water and littoral morphotypes. The open-water morphotype taken from Cowichan Lake by midwater trawl during winter seems to be entirely dependent on plankton (Carl 1953).

Benthic Foraging Trials

Two sets of experiments were conducted to test the foraging efficiency of the different morphotypes feeding on an experimental benthic prey.

Prey size experiments

These experiments were designed to test the hypothesis that the littoral morphotype, with its longer upper jaw, should be able to ingest larger prey items than the open-water population. The functional relationship between upper jaw length and maximum ingested prey size was tested by presenting brackish water amphipods (*Eogammarus confervicolus*) to each of the three populations. Individual fish were held in 20-L aquaria for 3 d prior to each test and fed *Eogammarus*. The bottom of each test aquarium was painted a uniform brown and individual aquaria were separated by partitions. Fish were starved for 24 h preceding each test. Periods of starvation longer than 24 h influence feeding behaviour in *Gasterosteus* (Beukema 1968).

Amphipods were anaesthetized with carbonated water and measured with an ocular micrometer. Each amphipod was assigned to a size category based on body length. Body length was defined as the distance from the base of the antennae to the base of the uropods with the body flexed (Bentzen 1982). Sixteen size-classes were tested ranging from 1.55 to 13.17 mm; size-class divisions were 0.77 mm. Amphipods were allowed to recover fully before introduction into the experimental aquaria.

Three amphipods, one from each of three consecutive size-classes, were presented to each fish. Fish were allowed 1 h to ingest the prey, after which the remaining amphipods were removed, reanaesthetized, and remeasured. After each trial, fish were fed to satiation with chopped liver. Following a further 24-h period of starvation, the test was repeated, but each of the three size-classes presented was increased by one division. Hence, the middle size-class of the preceding trial became the minimum size-class of the present trial, while the maximum size-class was increased by 0.77 mm. This sequence was continued until the individual could no longer ingest the largest amphipod of the three size-classes presented. If, after three consecutive trials, the fish had still failed to ingest this maximum size-class, the test was discontinued and the individual removed to measure its upper jaw length. Data were analysed by ANCOVA. In the initial analysis, standard length was treated as the covariate to determine the effects of upper jaw length on prey size, between morphotypes. This analysis was repeated substituting upper jaw length as the covariate. In the latter analysis, significant interpopulation differences in maximum ingested amphipod size imply the contribution of some effect other than upper jaw length.

Behavioural contributions to benthic foraging

A second series of experiments was conducted to examine the contribution of behaviour to interpopulation differences in benthic foraging success. Here, we have attampted to separate the contributions of behaviour and morphology on benthic foraging success. In these experiments, amphipods from a single size-class were presented to individual fish in a 218-L aquarium. The prey size-class chosen was 4.65 mm, which had been ingested by fish as small as 30.0 mm from all populations. Because some individuals take as many as 10 prey items during a 20-min feeding bout, 15 prey were presented to each fish. Fish were held individually in 20-L aquaria and starved for 24 h. Individuals were then placed in the experimental tank for 15 min preceding each test. This allowed time for acclimation to the tank. After 15 min the prey were introduced behind the fish from the top of the tank, and recording of the trial began after the first orientation on a prey item. The observer was positioned behind a blind and scored the number and duration of five behaviours: (1) orientation on a prey item, (2) strike on a prey item, (3) end of a successful manipulation, (4) end of an unsuccessful manipulation, and (5) a break in orientation with no strike at the prey. In none of the trials, each lasting 20 min, did any fish consume all prey. Data were recorded on an OS-3 event recorder (Observational Systems Inc.).

Interpopulation differences were investigated for several behavioural variables thought to contribute to benthic foraging success. These variables included the number of orientations on a prey item, the number of strikes at a prey item, the number of successful strikes at a prey item, the probability of a strike after orientation, and the average successful manipulation time. Average successful manipulation time is defined as the total time spent handling prey that were eventually ingested divided by the total number of prey ingested. Strike probability was expressed as the proportion of orientations that were followed by a strike. Differences between populations for each of these variables were tested using a Kruskal–Wallis one-way ANOVA on each variable.

The data were analyzed as a two-way fixed effects ANOVA on foraging success. The two factors in the ANOVA were population and upper jaw length. Population contained three levels, one corresponding to each morphotype, whereas upper jaw length contained two levels based on jaw lengths: $x < 3.8$ mm and $x \geq 3.8$ mm. Since upper jaw length is difficult to measure on live fish, the standard length of each test animal was measured and regression equations of upper jaw length on standard length were then used to assign individuals to one of the two levels of jaw length. Subsequent measures of upper jaw length on preserved individuals used in these trials indicated that this system produced no errors in classification. Foraging success was defined as the number of successful strikes (i.e. those followed by prey ingestion) divided by the total number of strikes. Probability plots (cumulative percent of the distribution versus raw data) indicated that foraging success had a non-normal distribution; consequently, the data were transformed using the arcsine square-root transform (Sokal and Rohlf 1981). At least 10 fish were tested within each cell. The ANOVA was computed using UBC:GENLIN (Greig and Bjerring 1980).

TABLE 1. Means and standard deviations (in parentheses) of size-adjusted data for each character and morphotype. All size-adjusted data log (base e) transformed. Letters in parentheses indicate no significant differences between means ($p < 0.01$). HEAL = head length; SNOL = snout length; EYED = eye diameter; UPJL = upper jaw length; GRL = gill raker length; GRN = gill raker number; HEAD = head depth; INOW = inner orbital width.

Character	Morphotype		
	Intermediate (a)	Open-water (b)	Littoral (c)
	Wild samples		
N	30	30	30
HEAL (ac)b	2.52 (0.092)	2.46 (0.036)	2.53 (0.053)
SNOL (abc)	1.29 (0.065)	1.29 (0.068)	1.28 (0.090)
EYED abc	1.34 (0.058)	1.37 (0.042)	1.40 (0.043)
UPJL (ab)c	1.11 (0.065)	1.07 (0.071)	1.22 (0.066)
GRL (ab)c	−0.23 (0.131)	−0.16 (0.126)	−0.37 (0.167)
GRN (ac)b	17.86 (1.130)	19.17 (1.080)	17.46 (0.860)
HEAD (ab)c	0.86 (0.071)	0.86 (0.067)	0.79 (0.076)
INOW (ac)b	2.88 (0.063)	2.98 (0.055)	2.86 (0.049)
	Laboratory progeny		
N	357	179	292
HEAL (abc)	2.54 (0.038)	2.56 (0.034)	2.56 (0.049)
SNOL abc	1.45 (0.061)	1.49 (0.067)	1.42 (0.062)
EYED (ab)c	1.39 (0.053)	1.39 (0.063)	1.41 (0.052)
UPJL (ab)c	1.13 (0.072)	1.13 (0.083)	1.17 (0.079)
GRL (ab)b	−0.06 (0.115)	−0.01 (0.120)	−0.07 (0.104)
GRN abc	19.25 (1.070)	20.34 (1.370)	18.14 (1.170)
HEAD abc	0.89 (0.068)	0.95 (0.060)	0.93 (0.061)
INOW abc	2.96 (0.055)	3.01 (0.077)	2.89 (0.065)

As all the fish in this experiment were capable of taking the size-class of prey presented, we predicted no difference in foraging success between populations. This assumed that the behavioural components to foraging success were approximately the same among populations. A significant effect of population, or a significant interaction term, would implicate some influence, other than jaw length, contributing to foraging success.

Limnetic Foraging Trials

If the greater number and length of gill rakers possessed by the open-water morphotype are the result of an adaptive divergence for feeding on limnetic prey, the limnetic foraging ability of the different morphotypes should differ in the laboratory. This hypothesis was tested using *Artemia salina* nauplii as the experimental limnetic prey. Particulate feeding teleosts tend to be size selective, and behaviour usually is a significant component in selectivity (O'Brien 1979). Hence, size-selective predation might obscure the significance of morphology, and for this reason only the first instar nauplii of *Artemia* were used. This instar is composed of a relatively constant size-class. In addition, nauplius colour appears to be approximately constant at this stage. Differences in prey colour can mediate differential attack responses in a variety of teleosts (review in Hyatt 1979).

Laboratory-reared fish were used in an attempt to standardize any learned component in interpopulation differences in limnetic feeding behaviour. All fish were initially reared on live *Artemia* nauplii and later switched to a mixture of liver and

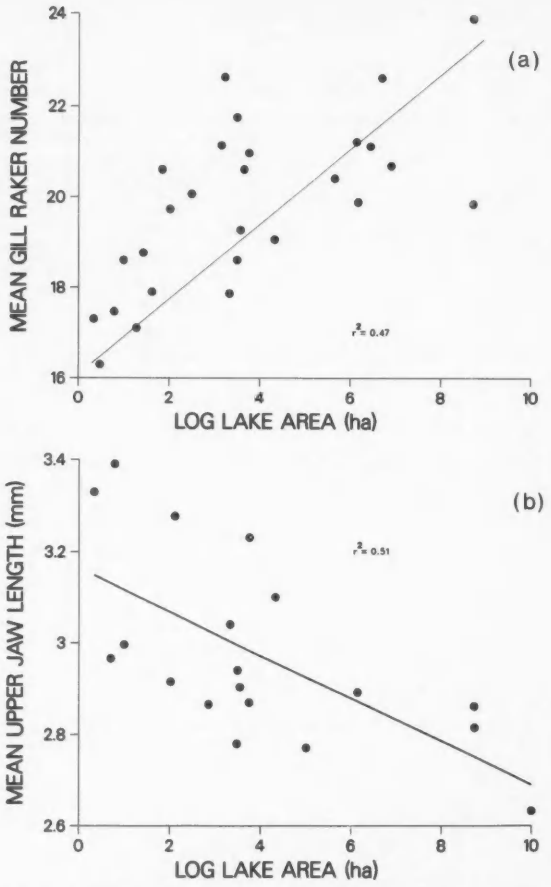

FIG. 1. Sample means related to lake area for lacustrine *Gasterosteus* populations within British Columbia. (a) Mean gill raker number; (b) mean upper jaw length size-adjusted to 40 mm within localities. (Lake names and locations may be obtained from authors.)

frozen *Artemia*. Test fish were chosen without bias from the tanks in which they had been reared.

Each test was run in the same 20-L aquaria used in the prey size experiments. To minimize suspended particles in the water, the tanks were scrubbed and refilled with dechlorinated water between each trial. Fish were held individually for 2 d before each test and fed to satiation on live *Artemia* nauplii. Tank temperature was 10 ± 0.5°C. At this temperature, total gut evacuation time in *Gasterosteus* is more than 16 h (Tugendhat 1960). To standardize hunger, individuals were starved for 24 h prior to each test. At the beginning of each trial, 100 *Artemia* (5 nauplii·L^{-1}) were presented to each individual. Fish were allowed to feed for 1 h and were then removed and sacrificed. Preservation was in 10% buffered formalin. After the fish had fixed for approximately 1 wk, their stomachs were excised between the upper and lower sphincters (Wootton 1976), opened, and the contents flushed with water. The number of *Artemia* nauplii per stomach was scored using a dissecting microscope. The nine morphological measures described in the "Trophic Morphology" section were made on each fish. Gill raker density was also estimated for each individual.

TABLE 2. ANCOVA results for gillraker density on standard length.

	Morphotype		
	Intermediate (N = 30)	Open-water (N = 30)	Littoral (N = 30)
Mean raker density	13.49	11.26	11.02
Adjusted mean raker density	14.16	12.03	9.42
SE of adjusted mean	0.42	0.48	0.49

Foraging success was expressed as the proportion of 100 prey taken by each fish. The data were arcsine square-root transformed (Sokal and Rohlf 1981) and interpopulation differences in limnetic foraging success tested using a one-way ANOVA. Univariate correlations were made within populations for each morphological variable against foraging success. All morphological variables, with the exception of GRN, were log (base e) transformed for all analyses.

Results

Trophic Morphology

The laboratory breeding study indicates a genetic component to each of the morphological characters scored. Interpopulation differences in character means are retained in laboratory-reared fish; however, there are four more significant (Scheffe's test, $p < 0.05$) character contrasts in the laboratory data than in the wild data (Table 1). This suggests that in wild fish, either selection or developmental plasticity or a combination of the two is masking some interpopulation genomic divergence.

Note that the littoral morphotype possesses a significantly longer jaw ($p < 0.05$) than either the intermediate or open-water population, but has significantly shorter gill rakers. In contrast, the open-water population has a significantly higher gill raker count. These appear to be general morphological patterns for lacustrine populations of *Gasterosteus* in British Columbia. As lake size increases, population mean gill raker number increases (Fig. 1a), while mean upper jaw length decreases (Fig. 1b). *Gasterosteus* populations from large open-water lakes feed almost exclusively on plankton, except during the breeding season when they move inshore (K. D. Hyatt, Pacific Biological Station, Hammond Bay Road, Nanaimo, B.C. V9R 5K6, pers. comm.). Both regressions on lake area are significant ($p < 0.01$) although the proportion of variance explained by each relation does not exceed 0.55.

For any given length, the intermediate and open-water morphotypes have more closely spaced rakers than the littoral populations (ANCOVA, $p < 0.001$, Table 2). Scheffe's test on the adjusted gill raker areas, indicates no differences between populations ($p > 0.10$). Thus, the littoral type has fewer rakers in the same relative space, than do the open-water and intermediate morphotypes.

Morphotypes Diet from Field Samples

Diet type, water column or benthic prey, was dependent on morphotype (chi-square, $p < 0.001$). Gut contents of the intermediate and littoral populations were dominated by chironomids and ostracods (Table 3). A small number of copepods were also found in the stomachs of the intermediate population, but no planktonic crustaceans were found in the stomachs of the littoral population. Diet of the open-water morphotype was dominated numerically, by water column and surface prey, although chironomids, and ostracods also contributed to their diet. Hence, the observed diets of stickleback morphotypes in the field are consistent with predictions based on population trophic morphology.

TABLE 3. Gut contents data from samples recovered in May 1983. Tabulated values are the pooled number of prey items per stomach for each sample.

	Morphotype		
Food item	Intermediate (N = 30)	Open-water (N = 20)	Littoral (N = 30)
Benthic			
Chironomids	103	12	128
Chaoborus			1
Megaloptera (larvae)			1
Megaloptera (adult)			1
Ephemeroptera	4		5
Unidentified insect (larvae)	4		4
Gasterosteus eggs	6	28	99
Unidentified eggs			10
Ostracods	312	20	433
Hydracarina			1
Nematodes	1		5
Gammaridae	2		
Water column			
Simulidae (adult)	2		8
Tipulidae (adult)	1		
Unidentified insect	2	6	3
Cladocera		26	
Cyclopoid copepods	2		
Calanoid copepods	2	68	

Benthic Foraging Trials

Prey size experiments

The littoral population ingested larger benthic prey items than either the intermediate or open-water population (ANCOVA, $p < 0.001$, Table 4). For both covariates, standard length and upper jaw length, the relationship with maximum ingested amphipod length was curvilinear. Therefore, all data were log (base e) transformed. None of the regression coefficients of amphipod length on standard length differed among morphotypes, but all were significantly different from zero (ANCOVA, $p < 0.05$). This indicates that within all morphotypes, larger fish can ingest larger prey items. For any given standard length, however, the littoral population, with its longer jaw, ingests significantly larger prey than either the intermediate or open-water population (Table 4a). Maximum prey size clearly increases as upper jaw length increases for all populations (Fig. 2).

Consequently, if we adjust maximum ingested prey size by upper jaw length, the resulting adjusted values should be the same among populations. This assumes that differences in ingested prey size are due solely to interpopulation differences in jaw length. This hypothesis was tested using upper jaw length as the covariate in the ANCOVA. Individual population regression slopes were not significantly different from one another ($p > 0.05$), nor was there any difference in the adjusted

TABLE 4. ANCOVA results for maximum ingested amphipod size on on (a) standard length and (b) upper jaw length.

	Morphotype		
	Intermediate	Open-water	Littoral
(a) Standard length			
Mean amphipod size	1.65	1.62	1.87
Adjusted mean amphipod size	1.63	1.56	1.94
SE of adjusted mean	0.06	0.07	0.06
Note: probability of equal adjusted means between morphs = 0.0007			
(b) Upper jaw length			
Mean amphipod size	1.65	1.62	1.87
Adjusted mean amphipod size	1.71	1.63	1.81
SE of adjusted mean	0.05	0.06	0.06
Note: probability of equal adjusted means between morphs = 0.1190			

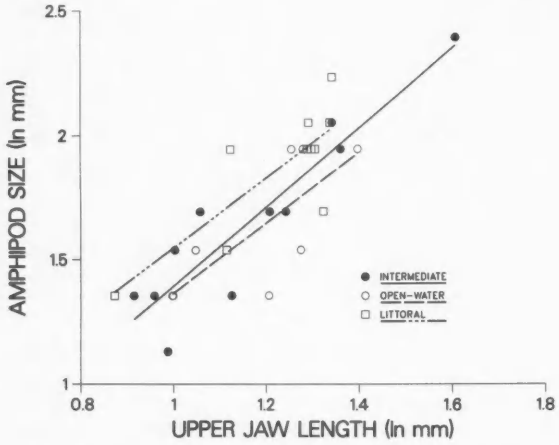

FIG. 2. Maximum size-class of amphipod ingested as a function of individual upper jaw length for each morph.

maximum ingested prey size between populations (Table 4b). Therefore, differences in maximum ingested prey size between morphotypes appear to be entirely attributable to interpopulation differences in upper jaw length.

Behavioural contributions to benthic foraging

There was no difference in foraging success among populations (ANOVA, $p > 0.05$); however, individuals with longer jaws had greater foraging success than smaller fish ($p < 0.001$). The interaction term (population × upper jaw length) was not significant. This result confirms the effect of upper jaw length on benthic foraging detected in the prey size experiments; as jaw length increases, benthic foraging ability also increases. Our inability to identify any difference in foraging success among morphotypes may result from individual variability in foraging behaviour. The large standard deviations

TABLE 5. Cell means and standard deviations (in parentheses) for ANOVA on benthic foraging success.

	Morphotype		
UPJL (mm)	Intermediate (N = 31)	Open-water (N = 21)	Littoral (N = 18)
<3.8	0.21 (0.28)	0.13 (0.23)	0.26 (0.31)
>3.8	0.48 (0.27)	0.54 (0.32)	0.54 (0.22)

TABLE 6. Multiple regression analysis using arcsine-transformed proportions of benthic foraging success as the dependent variable. Note: multiple $R = 0.61$ ($N = 70$).

Variable	Beta-weight	SE	Significance
Upper jaw length	0.39	0.09	$p = 0.0001$
Average successful manipulation time	0.41	0.09	$p = 0.0002$

TABLE 7. Sample means and standard deviations (in parentheses) from limnetic foraging experiments.

Morphotype	N	Mean number of prey taken	Mean open-water foraging success[a]
Intermediate	30	30.56 (21.16)	0.55 (0.26)
Open-water	31	31.93 (22.15)	0.56 (0.27)
Littoral	33	18.03 (18.45)	0.36 (0.28)

[a]Expressed as a mean proportion of the population's limnetic foraging success.

associated with benthic foraging success (Table 5) suggest that differences in individual behaviour may obscure interpopulation differences. Aside from individual differences, there may also be more general interpopulation differences in foraging behaviour that contribute to benthic foraging success.

This conclusion was supported by investigation of the behavioural variables, none of which differed among morphotypes, with the exception of average successful manipulation time (Kruskal–Wallis ANOVA, $p < 0.01$). Although the proportion of prey consumed did not differ among populations, littoral individuals appeared to spend less time manipulating prey before ingestion than the other two morphotypes. To address the relative contributions of manipulation time and jaw length to foraging success, both variables were entered into a multiple regression. Jaw length and manipulation time were the independent variables, and are of mixed mode; therefore, the data were standardized and the coefficients of regression become beta weights which are directly comparable (Sokal and Rohlf 1981). The overall regression was significant ($p < 0.001$), and both upper jaw length and average successful manipulation time had partial regression coefficients (Table 6) that contributed significantly to the model ($p < 0.001$). Hence, both upper jaw length and manipulation time contribute to benthic foraging success. The shorter prey handling time exhibited by littoral individuals is consistent with the prediction that it should be a superior benthic forager.

Limnetic Foraging Trials

Individuals from the littoral population were poor limnetic foragers when compared with the mean foraging success of both the intermediate and open-water samples (Table 7).

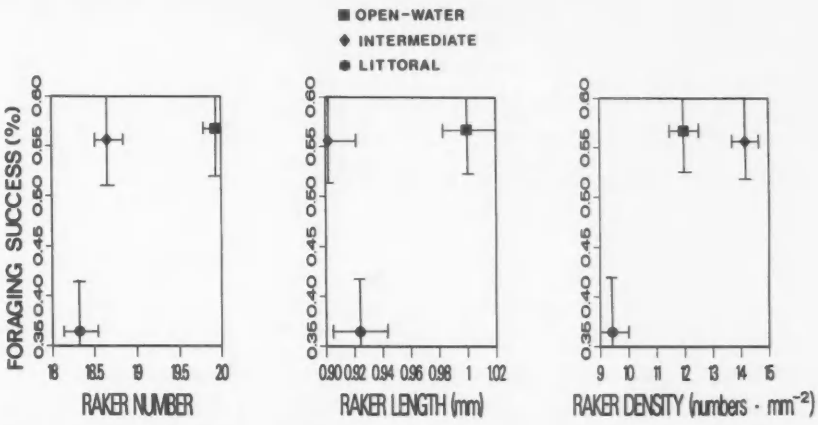

FIG. 3. Bivariate means for the proportion of limnetic foraging success and gill raker length, gill raker number, and gill raker density. Symbols indicate mean position for each population; bars indicate 1 SE on either side of the mean.

Sample means were heterogeneous among morphotypes (ANOVA, $p = 0.005$). Scheffe's contrasts indicated no significant differences between the open-water or intermediate individuals; however, individuals from both populations had significantly higher foraging success than the littoral population (Fig. 3, $p = 0.005$). All intrapopulation correlations of foraging success with morphological traits were nonsignificant (Pearson's r, $p > 0.05$). Within each population, however, functional relationships may be obscured by two factors: (1) the limited size range of individuals tested for each population and (2) individual behavioural variation. In each population a small number of fish appear to do either extremely poorly or extremely well on *Artemia*.

Discussion

Our results indicate that the differences in trophic morphology among populations in the Cowichan drainage are sufficient to produce detectable differences in foraging success on a given prey type. This result supports the hypothesis that interpopulation differences in trophic morphology are adaptive and evolved as responses to the primary prey types available in each lake (i.e. small, limnetic prey in large, oligotrophic lakes and larger, benthic prey in shallow, eutrophic lakes). Additionally, our results imply that the population genomes in the different lakes have undergone some selective divergence. If hybridization and gene flow occur among the different populations, the resulting offspring must be selectively eliminated. Gene flow should erode genetic divergence. Consequently, if gene flow is important in the Cowichan system, but its effects are obscured every generation by selection, then unselected progeny reared under identical laboratory conditions should not show the character differences typical of the adults. Since laboratory-reared progeny do show the same differences as wild adults, population genomes clearly have diverged. Each population then constitutes an ecological race (sensu Dobzhansky 1970). In addition, because the interpopulation genetic differences in trophic morphology appear to result from adaptive divergence (for jaw length and gill raker number), each morphotype may be considered an "ecotype" (sensu Turesson 1922).

The contribution of trophic phenotype to foraging success has been examined previously in *Gasterosteus*, but only in interspecific comparisons. Larson (1976) and Bentzen and McPhail (1984) demonstrated that differences in maximum ingested pairs (occurring in Enos Lake, Vancouver Island) were functions of differences in mouth gape. In these studies, the morphological differences were more pronounced than in our ecotypes and these sympatric pairs behave as biological species (McPhail 1984). Unfortunately, these sympatric species pairs may represent something of an evolutionary anomaly. The species pairs in Enos Lake are thought to have arisen through the double (or multiple) invasion of the lake during Pleistocene episodes of ice advance and retreat (McPhail 1984). Hence, genomic differences between species pairs may have arisen during the 500–1000 yr separating the last two saltwater intrusions (Mathews et al. 1970) of Enos Lake. Thus, it is difficult to evaluate the selective role of trophic resources in the divergence of morphology by generalizing from these species pairs. The present study, however, suggests that diet type may be a significant selective force in different populations, and hence responsible, at least in part, for the patterns of polytypic trophic variability observed across *Gasterosteus* populations in both North American (e.g. Hagen and Gilberton 1972) and Europe (e.g. Gross and Anderson 1984). This conclusion is supported by the pattern of increased upper jaw length with decreased lake size for lacustrine *Gasterosteus* populations from British Columbia. Secondary production in small, shallow lakes tends to be associated primarily with benthos (Northcote and Larkin 1956), and both the abundance and diversity of macroinvertebrates increase in such lakes; hence, selection favours individuals capable of consuming these larger prey items.

The littoral form tends to have a lower handling time. This should provide an energetic advantage and thus be selectively favoured (Schoener 1971); however, it may be unreasonable to expect to separate the individual contributions of behaviour and morphology to foraging success. A genetic correlation between morphology and behaviour would result in a correlated response to selection and thus the possible evolution of a "coadapted trophic character," comprising aspects of both morphology and behaviour. Possibly, interpopulation differences in handling time also arise through the covariance of this char-

acter with some unmeasured behavioural or morphological trait. Nevertheless, both the decreased prey manipulation time and greater jaw length are in accord with the diet of the littoral ecotype, and will therefore be selectively favoured.

In contrast with the littoral ecotype, the open-water ecotype is a superior forager in experiments conducted with water column prey. The differences in limnetic foraging ability among populations are therefore congruent with the observed interpopulation divergence in gill raker morphology. Certainly the largely planktonic diet of the open-water morphotype should select for characters that contribute to feeding success on planktonic prey.

Gill raker morphology may set a lower limit on the size of particles that can be retained (Hyatt 1979). The probability that a plankter will escape after passing into the buccal cavity is thought to be a function of gill raker retention (Drenner et al. 1978). Hence, high gill raker density should enhance plankton feeding, and numerous studies draw correlations between gill raker architecture and planktivory (e.g. Kliewer 1970; Magnuson and Heitz 1971; Seghers 1975; Wright et al. 1983). Unfortunately, the mechanism of gill raker action is still imperfectly understood. In some teleosts, particularly "filter feeders" (e.g. *Polyodon spathula*), gill rakers may act as a passive sieve. In these instances there may be a direct relation between spacing and retained particle size. For *Gasterosteus* and other particle feeders, however, the role of gill rakers is more complex. Typically, retained particle size is somewhat greater than minimum spacing (Wright et al. 1983); here again, behaviour seems to modify morphological constraints. By using only one size-class of *Artemia* nauplii, one should control for aspects of size selectivity and be able to identify a "step" in gill raker composition at which many fewer plankton are retained. That is, some point must exist at which minimum spacing exceeds the maximum size dimension of the nauplii. Intrapopulation variation may not contain such a step, which would account for the lack of correlation between foraging success and gill raker characteristics within populations. Interpopulation contrasts, however, do contain significant differences in raker morphology and the littoral population, with reduced gill raker density, is a significantly poorer planktonic forager than either the intermediate or open-water population. Again, the general pattern of increasing gill raker number with increasing lake size suggests that diet type is a common selective force organizing trophic morphology in *Gasterosteus*.

We were unable to determine whether the intermediate morphology of Bear Lake fish was translated into an intermediate foraging efficiency. This may result in part from the characters tested. Although, in terms of total morphological distance, the intermediate is more closely linked to the littoral population (Lavin and McPhail 1985), its upper jaw length was not significantly different from the open-water sample. For upper jaw length, then, the benthic foraging success of the intermediate ecotype is in the predicted direction. This is also true for its limnetic foraging success. The relatively efficient feeding on *Artemia* observed in the intermediate and open-water populations is associated with their high gill raker densities. The gill raker morphology of the intermediate may be a consequence of a variety of trophic selective pressures operating within generations. Lacustrine populations of *Gasterosteus* often move out of littoral regions after breeding. This habitat shift is often accompanied by a dietary switch to limnetic prey (Gross and Anderson 1984). Although Bear Lake contains an extensive littoral zone, there is a much larger pelagic region in which *Gasterosteus* must contact plankton. In Bear Lake the breeding season lasts for no more than a month and a half; hence, the population spends the majority of its life in a pelagic environment. In contrast, Grant Lake contains no appreciable pelagic zone and therefore this population is in constant contact with benthic prey.

If interpopulation selection on trophic morphology has led to ecotypic divergence as this study indicates, the mechanism(s) responsible for the maintenance of these differences is of primary interest. Once two forms establish divergent habitat preference the framework is established for incipient isolation (Mayr 1963). Geographic distance alone may be responsible for the maintenance of population identity between the littoral and open-water forms in this study; however, the proximity of the intermediate (Bear Lake) and open-water (Cowichan) forms precludes distance as an isolating mechanism. In this instance some degree of habitat selection and/or mate choice (or some combination of these two) are likely operating to maintain racial distinction, as the breeding seasons of the two forms are concurrent.

Hagen (1967) demonstrated that the freshwater and anadromous forms of *Gasterosteus* prefer different microhabitats for breeding, and Hay and McPhail (1975) also showed that these forms exhibit positive assortative mating that is based, at least in part, on male choice (McPhail and Hay 1983).

As yet, we have no data on assortative mating between ecotypes; however, the investigation of this possibility may provide insights into the origin of reproductive isolation between sympatric species pairs of *Gasterosteus*. It would be of interest to know whether selection maintains racial distinction after secondary contact, or whether habitat selection has led to premating isolating mechanisms. Certainly many workers have demonstrated that isolation may arise as a pleiotropic response to morphological shifts under contrasting selective regimes in the laboratory (e.g. Dobzhansky and Pavlovsky 1971; Van Dijken and Scharloo 1979). Gross changes in morphology, however, need not result in reproductive isolation. Sage and Selander (1975) suggested that radiation of trophic morphs may be achieved through polymorphism rather than speciation. A similar conclusion was reached by Turner and Grosse (1980) for differentiation in *Ilyodon*. Clearly, future research must focus on assortative mating between trophic morphs at some zone of contact. Such studies may ultimately identify the mechanism(s) by which racial integrity in *Gasterosteus* is maintained.

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Seasonal Dynamics of Phosphorus Partitioning and Export in Two Streams in Alberta, Canada

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Munn, N., and E. Prepas. 1986. Seasonal dynamics of phosphorus partitioning and export in two streams in Alberta, Canada. Can. J. Fish. Aquat. Sci. 43: 2464–2471.

In 1983, phosphorus (P) export was quantified for two streams in Alberta, Canada. The influence of changes in discharge on P concentration and partitioning was examined on an annual and seasonal basis and these data were used to develop empirical models to predict P concentrations. Phosphorus export peaked during summer storms; 68% of annual total P loading was transported during 12 d in early summer. Phosphorus increases were larger during storms in early summer than during storms in late summer. Annual total P exports were 7.5 and 13.0 mg/m^2 (watershed area) for the two streams, primarily in the fine particulate fraction. The empirical models presented are the first based on detailed data from watersheds with the following characteristics: (1) trees predominantly coniferous, (2) deep glacial till overlying sedimentary bedrock, and (3) climatic regime where most P is exported during summer storms. These results underscore the need for intensive sampling regimes in streams in a variety of regions (especially at high discharge) so factors controlling P levels can be better understood.

En 1983, la migration du phosphore (P) a été quantifiée dans deux cours d'eau de l'Alberta, au Canada. L'influence des modifications du débit sur les teneurs en P et sur sa séparation a été examinée sur une base annuelle et saisonnière et ces données ont servi à élaborer des modèles empiriques pour prévoir les teneurs en P. La migration de P a culminé pendant les tempêtes estivales; 68 % de la charge annuelle totale de P était transportée pendant 12 j au début de l'été. Les augmentations de phosphore étaient plus importantes pendant les tempêtes du début de l'été que pendant celles de la fin de l'été. Les taux annuels totaux d'exportation de P étaient de 7,5 et 13,0 mg/m^2 (superficie du bassin versant) pour les deux cours d'eau, principalement dans la fraction particulaire fine. Les modèles empiriques présentés sont les premiers basés sur des données détaillées provenant de bassins versants présentant les caractéristiques suivantes : 1) arbres regroupant surtout des conifères, 2) till glaciaire profond recouvrant une assise rocheuse sédimentaire et 3) régime climatique où la majeure partie de P est exportée pendant les tempêtes estivales. Ces résultats soulignent la nécessité d'entreprendre des programmes intensifs d'échantillonnage dans des cours d'eau de diverses régions (notamment où les débits sont élevés) afin de pouvoir mieux connaître les facteurs déterminant les niveaux de P.

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A decade ago, many studies were conducted to evaluate the effect of land use and geology on phosphorus (P) loading in different biomes (e.g. Dillon and Kirchner 1975; Omernik 1977; Rast and Lee 1978). In these projects, streams were sampled once every 2–3 wk. More recently, Stevens and Smith (1978), Meyer and Likens (1979), and Rigler (1979) demonstrated that most P export values reported in the literature are underestimates of actual loading; data derived from sampling regimes of 2 to 3 wk intervals are inadequate because they miss dramatic short-term changes during spring and storm runoff. However, these three intensive studies were conducted on watersheds situated on granitic bedrock; the importance of intensive streamwater sampling during high flows has never been evaluated for watersheds situated on sedimentary bedrock.

The transport of P within different size fractions and the relationship with discharge has been studied for two groups of streams flowing over granite bedrock: the headwater streams located in a deciduous forest at Hubbard Brook (Meyer and Likens 1979) and the grazed moorland streams in Dartmoor, England (Rigler 1979). In the Hubbard Brook and Dartmoor streams, increases in P concentration during floods were larger during the ascending than descending stage of the hydrograph, and P increases were greater in summer than in winter. In the moorland streams, total dissolved P (TDP; <0.45 μm) concentrations increased slightly when discharge increased whereas in Hubbard Brook, TDP levels did not change significantly when discharge increased. The contribution of coarse particulate P (CPP; >1 mm) to P loading and the variation in P concentrations between summer and winter have been investigated for only one stream (Meyer and Likens 1979).

Phosphorus export data are not available for streams running through glacial till, where groundwater seepage may be important, where seasonal discharge patterns vary from the classical spring and autumn peaks, and which are situated in drainage basins populated by coniferous rather than deciduous trees. This information is necessary both to develop a broader perspective on seasonal patterns in P loading and partitioning in streams and to monitor P export from similar streams in the boreal forest. Thus, in two undisturbed streams located on glacial till in a boreal biome in central Alberta, we investigated

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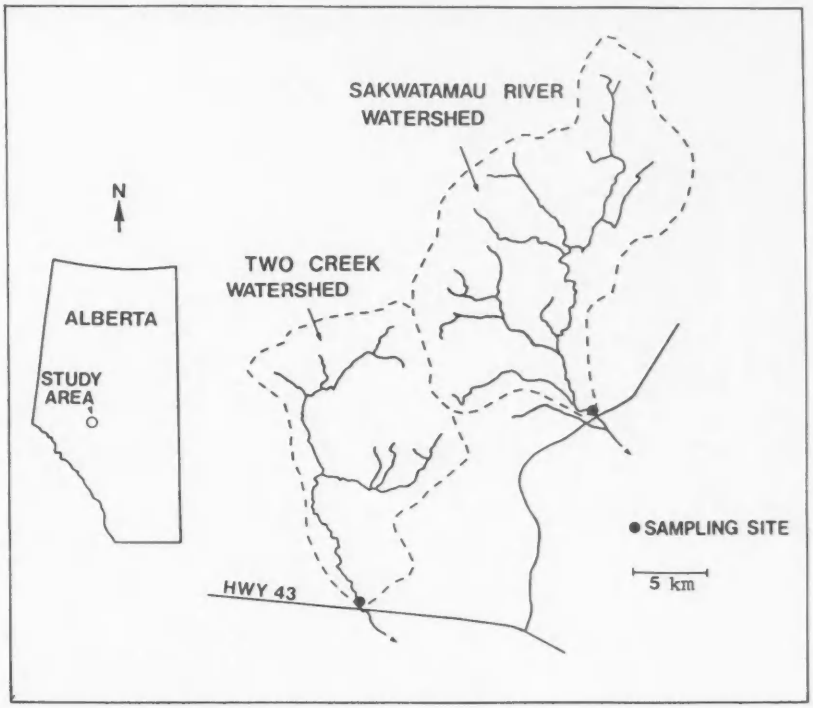

FIG. 1. Map of study area.

(1) the influence of season on P export and (2) the influence of deterministic (e.g. spring runoff) and stochastic (e.g. storms) changes in the ecosystem on P concentration and partitioning. We also developed models to predict P concentration in these forested streams and evaluated previously published expressions for P export.

Site Description

We conducted this study in 1983 on two adjacent forested watersheds (Two Creek and Sakwatamau River) located 230 km northwest of Edmonton, Alberta (Fig. 1). The most recent disturbance in the watersheds was a fire in the late nineteenth century. At the sampling sites, Two Creek (54°18′N, 116°20′W) is a third-order stream draining 161 km^2 and Sakwatamau River (54°24′N, 116°05′W) is a fourth-order stream draining a 281-km^2 watershed. Two Creek and Sakwatamau River drain into the Athabasca River 8 and 30 km downstream from the sampling sites, respectively. Drainage densities are 0.34 and 0.37/km for the two streams. Debris dams are rare; intrusions within 3 km of either study site never block more than half of the stream channel. Maximum water depth at the study sites ranged between 8 cm in autumn to over 2 m in late June. Bryophytes were never present in the stream channel and periphyton growth was observed only during a 2-wk period in early autumn.

Vegetation is similar in both watersheds. The area is mixed boreal forest dominated by white spruce (*Picea glauca*), lodgepole pine (*Pinus contorta*), aspen poplar (*Populus tremuloides*), and balsam poplar (*Populus balsamifera*). Shrub species along stream banks include willows (*Salix* spp.), wild rose (*Rosa acicularis*), river alder (*Alnus tenufolia*), and various berries. Stream faunal communities are represented by invertebrates, mountain whitefish (*Prosopium williamsoni*), and arctic grayling (*Thymallus arcticus*).

Bedrock underlying the streams is sedimentary (shale, sandstone, and mudstone) of the Paskapoo formation (Paleocene) overlain by 3–5 m of glacial till (Knapik and Lindsay 1983). Watershed soils are primarily Gleysolic, which is a clay or sandy-clay loam, and soils along the stream beds are high in organic content (Knapik and Lindsay 1983).

Dominant ions in the stream water were calcium and bicarbonate; average conductivity was moderate (185 and 174 μS/cm for Two Creek and Sakwatamau River, respectively). Turbidity of streamwater was low (2.5 and 2.6 NTU for Two Creek and Sakwatamau River, respectively) although colour was moderate (69 and 47 mg Pt/L for Two Creek and Sakwatamau River, respectively). Total nitrogen to total P ratios were moderate (22 to 27 by weight). In both streams, pH was high (8.1) and water temperature ranged between 0 and 20°C (Munn 1984).

Materials and Methods

Field Sampling

We conducted field work from stream ice-off (April 22) to freeze-up (October 25) during 1983. Two Creek and Sakwatamau River were sampled at least twice daily except for sixteen 2-d periods when samples were being analyzed in the laboratory. During storms, sampling frequency was increased and corresponded to the time interval necessary for gauge height to

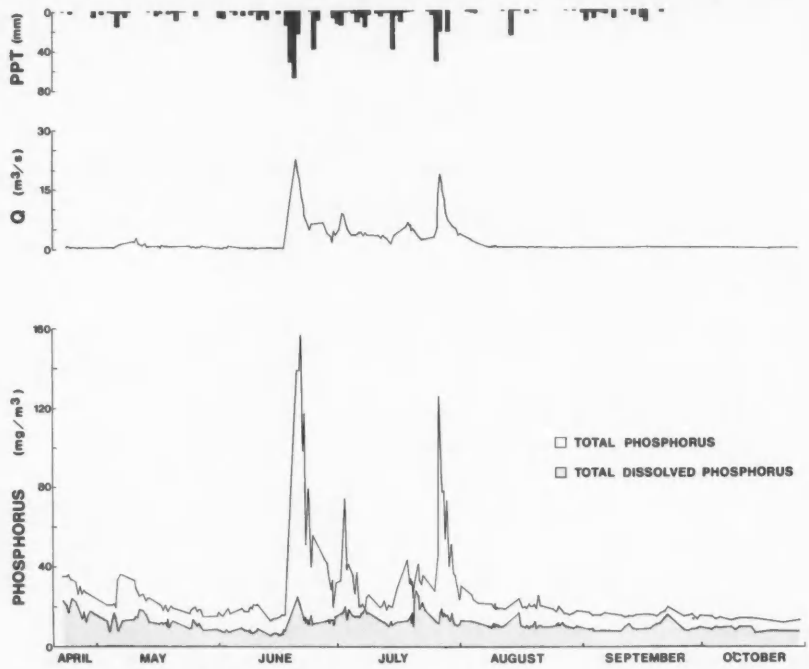

FIG. 2. Variation in precipitation (PPT), discharge (Q), total phosphorus (TP), and total dissolved phosphorus (TDP) concentrations from April to October 1983 for Two Creek.

change by 3 cm, or if gauge height changed slowly, sampling frequency was once per hour. We used a Price AA current meter to measure velocity and water depth at 1.0- or 0.5-m intervals across the stream width. Water level was recorded with continuous recorders and with depth gauges placed in each stream in April. On each visit, we collected a grab sample of streamwater from the middle of each stream (at 0.7 times maximum water depth below the surface) in 1-L Nalgene bottles for P analysis. Stream water to be analyzed to total P (TP) was immediately filtered through a 1-mm mesh net and placed into 50-mL culture tubes. Water to be analyzed for TDP was filtered through a prerinsed 0.45-μm Millipore HAWP membrane filter and subsamples were also poured into culture tubes. Fine particulate P (FPP) was later calculated as the difference between TP and TDP. To quantify more precisely the size of materials within the fine particulate fraction, FPP was divided into five subclasses by filtration (1 mm − 80 μm, 80−64 μm, 64−10 μm, 10−5 μm, and 5−0.45 μm) on eight dates from August to October as well as on three dates in the spring of 1984.

To estimate transport of CPP (>1 mm), we collected coarse particulate matter (CPM) with a 1-mm mesh net (35 × 40 cm) placed in each stream. To quantify the volume of water passing through the nets, water velocity was measured 1 m in front of each net at the time of sampling. Material was collected daily from June to October and later analyzed for P content.

To test if one grab sample was sufficient to estimate P transported downstream, replicate grab samples were taken monthly from both streams. We tested spatial heterogeneity across the stream with grab samples taken at 2-m intervals across the width of the stream and analyzed for TP and TDP concentration. To test for diel variation in FPP and TDP concentrations, we collected grab samples of streamwater every 4 h for 24 h on five dates for Two Creek and on four dates for Sakwatamau River.

We obtained daily precipitation data from an Alberta Forest Service fire lookout station located on the northern edge of Two Creek watershed. Discharge data collected for Sakwatamau River in 1983 were compared with discharge data collected by Environment Canada since 1973 at the mouth of Sakwatamau River (Environment Canada 1973−83). For this study, we defined a storm as any rain event that resulted in at least a 5% increase in discharge over prerain discharge levels.

Heavy rainstorms flooded the road to Sakwatamau River from July 4 to 19 (2 wk after peakflow) and the stream could not be sampled. Consequently, Sakwatamau River loading data were interpolated from a regression of Sakwatamau River P loading data on Two Creek P loading data for the summer months excluding this 16-d period (r = 0.92). Sakwatamau River data were not used to analyze the influence of July storms on P concentration and partitioning. During they heavy rain storms in July, water depth increased in both streams such that normal sampling procedures were disrupted; flow velocity was calculated for this period by multiplying surface velocity by a factor of 0.8 (Gray 1970). To collect CPM during high water levels, 1-mm-mesh nets were held in the water for 3−5 min from a position on the streambank.

The two study streams were frozen to the bottom from November to April and consequently there was no P export during this period.

Laboratory Methods

Total P, TDP, and FPP concentrations were determined in

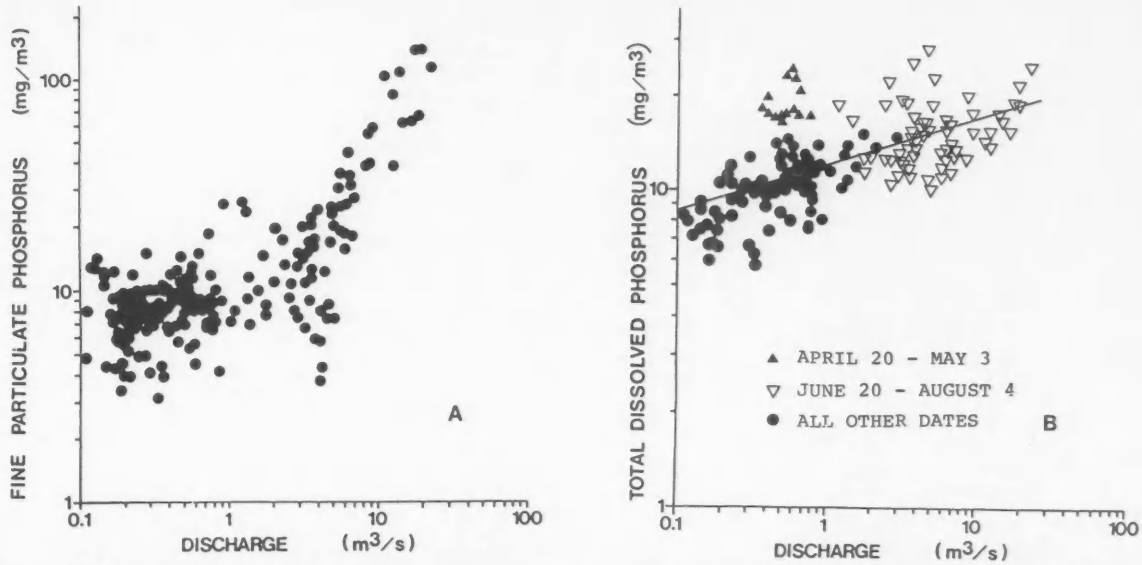

FIG. 3. Empirical relationships between (A) fine particulate phosphorus concentration (FPP), discharge (Q), and rate of change of discharge (R; $m^3 \cdot s^{-1} \cdot h^{-1}$) in Two Creek and (B) total dissolved phosphorus concentration (TDP) and discharge. Fig. 3A. ln FPP = 2.12 + 0.29 ln discharge + 0.16(ln discharge)^2 + 0.04R (r = 0.81); Fig. 3B. ln TDP = 2.48 + 0.16 ln discharge (r = 0.68).

TABLE 1. Average total phosphorus (TP), fine particulate phosphorus (FPP), and total dissolved phosphorus (TDP) concentrations (mg/m^3) for Two Creek and Sakwatamau River in 1983.

	Two Creek			Sakwatamau River		
Month	TP	TDP	FPP	TP	TDP	FPP
April	28.4	17.5	10.9	37.0	21.7	15.2
May	22.0	11.6	10.3	22.7	12.7	10.1
June	43.3	11.6	32.2	48.3	11.0	38.2
July	35.0	14.7	20.3	32.3	23.3	13.1
August	20.5	11.2	9.4	18.0	10.7	7.4
September	15.8	9.3	6.6	14.6	9.7	5.0
October	13.5	8.6	4.9	13.6	9.2	4.4
Average	25.5	11.7	14.0	25.8	13.1	13.1

triplicate (SE always <7% of mean P value) on filtered subsamples and analyzed by the potassium persulfate technique (Prepas and Rigler 1982). Coarse particulate matter was dried for 24 h at 105°C and weighed. Approximately monthly, six 1-g subsamples of dried CPM were analyzed for P content and we used these data to convert daily CPM dry weights to daily CPP values.

Data Manipulation

We calculated P export values by summing the product of P concentration and discharge for each sampling interval. Relationships between TP, FPP, and TDP concentrations and discharge were analyzed on an annual and seasonal basis with regression and correlation analysis. While we recognize the probability of autocorrelation with time series measurements such as P concentration and discharge, correlation analysis between the measured variables provides valuable comparative information. Time series analysis is inappropriate because of irregular sampling frequency. To reduce the effect of outliers, these analyses were performed on log-transformed data. When the data were grouped seasonally or on the rising versus falling stage of the discharge hydrograph, analysis of covariance (ANCOVA) was used to test for equality of linear regression lines (slope and intercepts). Replicate grab samples were compared with paired t-tests. Data collected to estimate spatial heterogeneity as well as data to test for diel variation were analyzed with the maximum normed residual (MNR) test for outliers (Snedecor and Cochran 1980, p. 280). Since multiple comparisons were used when looking at diel variation and spatial heterogeneity, significance was accepted at $P < 0.01$. The MNR test is not refined enough to test for small differences; only large differences could be detected.

We used data from Two Creek for most illustrations except where important differences between the two streams warranted added illustrations.

Results

When replicate grab samples collected at the same time and location were compared, there were no significant differences in TP concentrations (16.6 ± 1.2 mg/m^3, n = 11, t = 1.82, $P > 0.05$) and TDP concentrations (10.2 ± 0.4 mg/m^3, n = 11, t = 0.75, $P > 0.4$) in either stream. Similarly, when samples collected across the width of either stream at the same time were compared, there were no differences in FPP or TDP concentrations on any of the dates sampled ($P > 0.05$). Even with significant differences in velocity across the width of Two Creek on three dates, P concentrations in the stream channel were uniform. Thus, the grab sample technique used in this study was adequate to estimate P concentration in streamwater. There were no diurnal changes ($P > 0.01$) in discharge, FPP, and TDP concentrations for Two Creek and Sakwatamau River based on eight 24-h sampling series which were storm-free. These results suggest no diel patterns in P concentrations in Two Creek and Sakwatamau River.

TABLE 2. Transport of fine particulate phosphorus (FPP) during spring melt expressed in mass (kg) and mass per unit area of watershed (mg/m^2).

Date	Two Creek		Sakwatamau River	
	kg	mg/m^2	kg	mg/m^2
April 23	0.94	0.006	7.41	0.026
24	0.58	0.004	2.39	0.008
25	0.43	0.003	0.97	0.003
26	0.40	0.002	3.04	0.011
27	0.49	0.003	3.35	0.012
28	0.53	0.003	2.31	0.008
29	0.29	0.002	0.94	0.003
May 3	0.42	0.003	0.88	0.003
4	0.49	0.003	1.39	0.005

From April 22 to October 25, 623 mm of precipitation fell on the watersheds, most (75%) during June and July (Fig. 2). Consequently, 82 and 64% of total annual discharge was measured during June and July for Two Creek and Sakwatamau River, respectively. This pattern of heavy rainfall during early summer is not unusual for the region; maximum daily discharge at the mouth of Sakwatamau River occurred during June in 5 of the 11 yr for which discharge data exist (Environment Canada 1973–83). Overland flow occurs at maximum instantaneous discharge greater than approximately 85 m^3/s. Based on maximum instantaneous discharge records, overland flow occurred at least four times during these same 11 yr. Thus, materials transported during overland flow could have accumulated in the watershed for several years between high flow periods before being transported downstream.

Since such a high percentage of annual rainfall fell during late June and July, discrete storm analysis was difficult, and cumulative effects of rainfall were very important (Fig. 2). During storms in June and early July, dramatic increases in all P fractions were recorded, even after peak rainfall. Consequently, P concentrations were higher on the falling limb rather than on the rising limb of the storm hydrograph; for example, TP concentration at the same discharge was 31.8 mg/m^3 on the rising limb and 35.9 mg/m^3 on the fallimg limb. However, during a storm in late July, increases in P concentration were smaller and P concentrations were higher on the rising limb of the hydrograph (clockwise hysteresis); for example, TP concentration at the same discharge was 47.1 mg/m^3 on the rising limb and 36.4 mg/m^3 on the fallng limb of the hydrograph.

Seasonal Patterns in Phosphorus Concentration

In both streams, mean monthly TP and FPP concentrations (weighted for sampling frequency) were highest in June when discharge was also high (Table 1). In Sakwatamau River, average monthly TDP concentration was highest in July whereas in Two Creek, TDP was highest in April. In both streams, TP, TDP, and FPP concentrations were lowest in October when discharge was also low. Thus, the seasonal patterns of TDP and FPP concentrations were similar in the study streams.

Average spring FPP concentration in Sakwatamau River was 39% higher than the average concentration for Two Creek (Table 1). This difference may reflect different melting patterns in the two streams. Ice on the Sakwatamau River streambed melted before April 22, and all ice along the streambank was gone by April 24. However, in Two Creek, ice melted from the top to the bottom and ice on the substrate did not melt until May 3. Transport of FPP was significantly greater in Sakwatamau River than in Two Creek from April 22 to May 3 (Wilcoxon two-sample rank test, $P < 0.01$, Table 2). During this 12-d period there was no resuspension of FPP from the streambed in Two Creek whereas there was resuspension from the streambed in Sakwatamau River. From April 22 to May 3, 21.3 kg (0.08 mg/m^2 of watershed) of FPP was transported in Sakwatamau River whereas only 4.1 kg (0.02 mg/m^2) was transported in Two Creek. If identical physical and chemical influences were operating on both streams during this period, then 81% (17.2 kg or 0.06 mg/m^2) of FPP transported on both streams during spring was resuspended from the streambed in Sakwatamau River. While it is unlikely that identical conditions exist in the two streams, these results emphasize the potential contribution of materials resuspended from the streambed to transported FPP during spring.

From August to October, most of the P in the FPP fraction was bound to materials in the 5–10-μm fraction (33% in Two Creek). This tendency toward transport of particulate P on small particles was more apparent in later autumn when flows were lowest. From August to October, the contribution of the 10 μm – 1 mm fraction was relatively small in Two Creek (18%) and Sakwatamau River (17%). However, from April to June 1984, no fraction of FPP was dominant when flows were high.

Empirical Models

Discharge and FPP concentration were strongly correlated ($r = 0.81$ for Two Creek, Fig. 3A). A log–log plot of concentration versus discharge is curvilinear described by a second-order polynomial regression with rate of change of discharge (R) as a third independent variable. No differences were apparent when the regressions (excluding R) were compared for the rising and falling stage of the hydrograph (ANCOVA; $n = 207$, $P = 0.12$ for Two Creek; $n = 165$, $P = 0.75$ for Sakwatamau River). In Two Creek, there were no significant seasonal differences in the relationship of FPP on discharge between early versus late summer storms ($n = 65$, $P = 0.08$); there were insufficient data to do a similar analysis for Sakwatamau River. In both Two Creek and Sakwatamau River, TDP concentrations were positively correlated with discharge ($r = 0.68$ and $r = 0.66$, respectively; Fig. 3B). For the TDP-Q data, a nonlinear model does not explain more of the variation in the data than the linear model. Finally, the relationship between TP concentration and discharge is curvilinear and described by a second-order polynomial ($\ln TP = 3.04 + 0.25 \ln Q + 0.09(\ln Q)^2 + 0.12R$, $r = 0.87$, $n = 209$). Regressions of TP on Q and R were described for data based on early summer storms (June 18 – July 13; $r = 0.94$, $n = 33$) versus late summer storms (July 14 – August 4; $r = 0.90$, $n = 33$). A difference in the regressions was apparent ($P < 0.001$) which may be related to the hysteresis effect observed during storms in the late summer period only.

The relationship between CPM and Q was positive, reflecting the strong increase in total CPM transport during high discharge ($r = 0.88$ and $r = 0.79$ for Two Creek and Sakwatamau River, respectively; Fig. 4). The mass of CPM transported downstream during storms was large; CPM transport was largest in June (25 918 and 4365 kg for Two Creek and Sakwatamau River, respectively) and lowest in October (3 kg for both streams). The seasonal patterns in P content of CPM were similar between streams; P content of CPM generally increased

TABLE 3. Mass of total phosphorus (TP), total dissolved phosphorus (TDP), fine particulate phosphorus (FPP), and coarse particulate phosphorus (CPP) transported past the Two Creek and Sakwatamau River sites during each month of the study and over the whole study period (1983) expressed in total mass (kg) and mass per unit area of water shed (export coefficient mg/m^2). Blanks indicate no samples taken.

	Two Creek				Sakwatamau River			
Month	TP	TDP	FPP	CPP	TP	TDP	FPP	CPP
April	11.1	6.7	4.4		53.1	29.9	23.2	
May	59.5	29.8	29.7		86.7	44.7	42.0	
June	1326.0	249.5	1076.5	33.69	1265.8	181.2	1084.6	4.80
July	584.4	201.2	383.2	30.43	556.8	191.2	365.6	0.83
August	37.5	19.5	18.0	0.09	76.4	42.4	33.9	0.13
September	11.3	6.6	4.7	0.01	37.7	25.2	12.6	0.02
October	5.4	3.4	2.0	0.00	22.8	15.4	7.4	0.00
Total transport	2035.2	516.7	1518.5	64.23	2099.3	530.0	1569.3	5.78
Export coefficient	12.64	3.21	9.43	0.40	7.47	1.87	5.58	0.02

over summer (from 0.13 to 0.21% of dry weight in Two Creek and from 0.11 to 0.22% in Sakwatamau River) and then declined in late autumn. However, 99 and 98% of annual CPM export for Two Creek and Sakwatamau River, respectively, was transported during storms in June and July, so CPP loading was highest in summer when flows were high and lowest in autumn when flows were low (Table 3).

Phosphorus Export

During 1983, 13.0 mg P/m^2 (watershed area) and 7.5 mg P/m^2 (sum of TP and CPP) was exported from Two Creek and Sakwatamau River, respectively. In both streams, 72% of the annual P export was in the FPP fraction. Coarse particulate P was relatively insignificant; CPP was 3 and 0.3% of total P transported in Two Creek and Sakwatamau River. In Two Creek, P transport was greatest in June for all three size fractions, and in Sakwatamau River the general pattern held, although TDP transport was also high in July. For all size fractions in both streams, P concentration was lowest during October. In all months except June and July, TDP transport was greater than FPP trasport. Thus, the pattern of P export was similar between the streams despite the difference in export coefficients.

Flow duration curves emphasize the relationship between storms and P export. For example, in Two Creek, 42% of the annual TP export was transported with 19% of the water in 2% of the time (Fig. 5A). Export of FPP was more strongly pulsed than was export of TDP; 75% of annual FPP export was transported within the same time period as 47% of TDP transport (Fig. 5B). To determine how important the two largest storms (June 17–21 and July 24–30) were in terms of pulsing, the mass of P transported during these storms was calculated. In Two Creek, 83% of the TP loading for June or 55% of the annual TP loading was transported during the first storm, and 50% of the TP loading for July or 14% of annual TP export was transported during the second large storm. Consequently, 68% of annual TP loading and 76% of annual FPP loading was transported during 12 d. Thus, P export, primarily in the FPP fraction, was strongly pulsed during storms.

Discussion

Although there was no relationship between TDP and discharge in the forested Hubbard Brook streams (Meyer and Likens 1979), there was a positive relationship between TDP and discharge in Two Creek and Sakwatamau River in grazed watershed in Dartmoor, England (Rigler 1979). These differences may be related to differences in stream size and watershed land use.

In Two Creek and Sakwatamau River, FPP concentrations increased as discharge increased. These results concur with those from Hubbard Brook (Meyer and Likens 1979) and Dartmoor (Rigler 1979) streams. However, these two earlier studies reported significant differences between FPP concentrations on rising and falling stages of the discharge hydrograph. In Two Creek and Sakwatamau River, there were no significant differences between FPP concentrations on the rising versus falling stage when all data are included in the analysis (excluding *R*). However, hysteresis effects were not observed during early summer storms but were observed in late summer storms. This suggests that the FPP pool accumulated during low flows the previous autumn and winter and the relatively long period between ice-out and the first summer storm. This accumulation was sufficiently large previous to early summer storms that is continued to contribute to P concentrations in the flowing water while discharge was declining. Hysteresis effects observed during late summer suggest that FPP pools in the stream channel were more quickly and/or easily depleted due to a shorter time period for accumulation of FPP. For example, based on days since equivalent discharge, there were 350 d for accumulation of FPP before the June 22 storm whereas there were only 34 d for accumulation before the July 26 storm. Thus, FPP transport is dependent on length of time since the last storm as well as on discharge. The dependence of FPP transport on discharge is further supported by our fractionation data; P was transported in all FPP fractions at high flows and mainly in smaller FPP fractions at low flows. This pattern of transport is consistent with the variation in sediment particle-size distribution in Ontario streams (Ongley et al. 1981).

In Two Creek and Sakwatamau River, P content of CPM increased during summer, peaked during early autumn, and then declined during late autumn. The peak in P content in early autumn is likely due to increased organic content and thus increased P content of CPM at low flows (Hobbie and Likens 1973; Meyer and Likens 1979). The decline in P content of CPM in late autumn in the study streams could be attributed to retention of nutrients by trees when they shed their needles or leaves. Even though leaffall on the watersheds was slight,

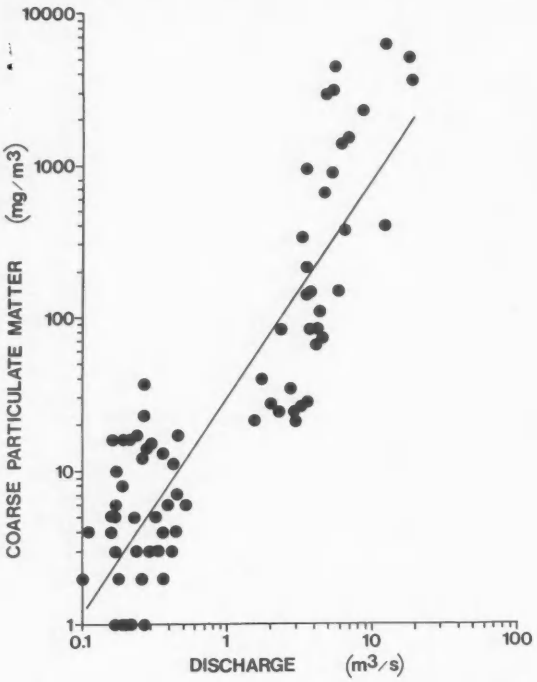

FIG. 4. Empirical relationship between coarse particulate matter (CPM; dry weight) transport and discharge in Two Creek (ln CPM = 3.47 + 1.38 ln discharge, $r = 0.88$).

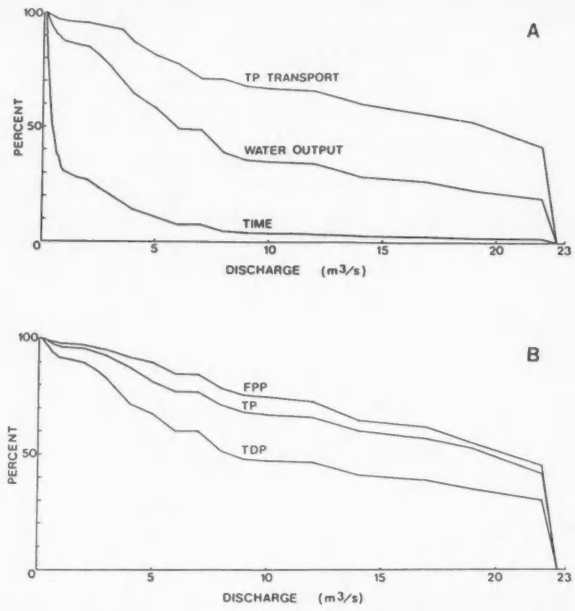

FIG. 5. Flow duration curves for Two Creek indicating (A) the percentage of total time, water output, and total phosphorus (TP) transport measured at or above the specified discharge and (B) the percentage of TP (as in Fig 5A), fine particulate phosphorus (FPP), and total dissolved phosphorus (TDP) transport measured at or above the specified discharge.

greater amounts of spruce needles were transported in the streams in late autumn when the P content of CPM declined. Overall, P transport in CPM was most important in summer because of the large mass of CPM transported during storms. Thus, the large fluctuations in discharge had a greater influence on P transport in CPM than did fluctuations in P content of CPM. We have no explanation for the difference in CPM transport in the two streams; however, the contribution of CPP to P export is relatively small for both streams.

We were surprised to find that the annual P export coefficient from Sakwatamau River was almost twice that from Two Creek (13.0 and 7.5 mg/m^2). The two watersheds have similar forest development, slope, soil characteristics, and drainage densities. Both stream channels have cobbled substrates with similar retention structures. Average monthly P concentrations, P mass exported, and water yields were similar for the two streams (Tables 1, 3). However, the Sakwatamau River watershed is 43% larger than the Two Creek watershed and thus the difference in export coefficients. The difference between the export coefficients for the two watersheds suggests that approaches to calculating export values in the literature may not be appropriate for streams on glacial till over sedimentary bedrock. One explanation for the discrepancy in export coefficients calculated in this study is that the hydrologic drainage area is much larger than that indicated by surface topography. An alternative model to predict P export is based on watershed drainage density and data from 18 streams in forested, igneous drainage basins (Kirchner 1975). This model is neither a good predictor of annual P export in our two study streams (3.1 and 3.2 mg·m^{-2}·yr^{-1} for Two Creek and Sakwatamau River, respectively) nor is it able to give any insight as to why the two measured export coefficients are so different. These results demonstrate that more carefully measured P budgets are needed for streams flowing through glacial till before factors controlling P levels in such streams can be better understood.

In the study streams, P transport was strongly pulsed during the first part of the summer and storm events were the dominant factor influencing P transport. Other studies report strong P pulsing, although sources and timing of the pulses vary. For example, in a forested stream in New Hampshire, 67% of the annual P exports were transported during 10 d, mainly in spring and autumn (Meyer and Likens 1979). In streams from Missouri to the Northwest Territories a major portion of P exports was associated with the first few days or even hours of spring runoff (Alberts et al. 1978; de March 1975; H. E. Welch, Freshwater Institute, Winnipeg, Man., pers. comm.). A low winter snowpack in central Alberta as compared with central and eastern Canada and the New England states, as well as a long period of snowmelt, could explain the negligible importance of spring runoff in this study. Regional differences in patterns of storms will influence P pulsing; in the Hubbard Brook watershed, major storms are more likely to occur in spring and autumn (Meyer and Likens 1979) whereas in central Alberta, major storms are more likely to occur in early summer. Thus, the timing of P pulses may be predictable on a regional scale based on climatic regimes, and climatic patterns should be the basis of programs designed to estimate annual P export (Meyer and Likens 1979). For example, in central Alberta, a majority of total P loading is associated with summer storms, and intensive sampling is required every few hours during these storms whereas daily sampling would suffice

during spring runoff and bimonthly sampling is adequate during low flow in the fall. When annual TP loading coefficients were recalculated for Two Creek based on samples collected once a week, the coefficients were underestimated by an average of 15% (range based on when the sample was taken: underestimate of 54% to overestimate of 10%), and for Sakwatamau River, the loadings were underestimated by an average of 34% (range: underestimates of 49 to 10%). Thus, P export coefficients can be severely underestimated with a weekly sampling program.

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Algal Picoplankton from Marine and Freshwater Ecosystems: A Multidisciplinary Perspective

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The discovery of minute (0.2–2.0 μm) algal picoplankton in the late 1970's in both marine and freshwater ecosystems has led to a resurgence of research activity throughout the world, addressing questions related to taxonomy, distribution and abundance, physiology and biochemistry, and ecological considerations of their role in aquatic food webs. To many, their discovery provided the "missing link" in the controversial carbon supply–demand question in the world's oceans and gave further credibility to the emerging new paradigm on the importance of microbial food webs in energy transfer and nutrient recycling in aquatic systems. In this essay we provide the first comprehensive multidisciplinary review of phototrophic picoplankton in lake and ocean ecosystems, discussing what is currently known about their occurrence, taxonomy, physiology, and biochemistry and their role in primary production and aquatic food webs.

La découverte, vers la fin des années 1970, d'algues picoplanctoniques (0,2–2,0 μm) dans des écosystèmes marins et dulçaquicoles a donné un nouvel essor, au niveau mondial, aux recherches portant sur la taxonomie, la distribution et l'abondance, la physiologie et la biochimie de ces algues, de même qu'à l'élaboration d'hypothèses sur leur rôle écologique dans les réseaux alimentaires aquatiques. Pour plusieurs, cette découverte constituait le « chaînon manquant » de l'équilibre controversé entre l'apport et la demande du carbone dans les océans et appuyait encore plus le nouveau concept accordant une grande importance aux réseaux alimentaires microbiens pour le transfert énergétique et le recyclage des matières nutritives dans les systèmes aquatiques. Les auteurs présentent la première analyse multidisciplinaire détaillée du picoplancton phototrophe dans les écosystèmes lacustres et océaniques. Ils traitent des connaissances actuelles touchant la présence, la taxonomie, la physiologie et la biochimie de ces organismes de même que de leurs effets sur la production primaire et les réseaux alimentaires aquatiques.

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I. Introduction: Definitions and Scope of Review

> Full many a gem of purest ray serene
> The dark unfathom'd caves of ocean bear;
> Full many a flower is born to blush unseen,
> And waste its sweetness on the desert air.
>
> — T. Gray, *Elegy*

It is true to say that the picoplankton lay mostly unseen or unrecognized in both lakes and oceans for several decades of scientific inquiry, until two oceanographic schools simultaneously announced their discovery of planktonic chroococcoid cyanobacteria, of cell dimensions approximately 0.5–1.0 × 1.0–2.0 μm, occurring widespread in all the seas and oceans examined (Johnson and Sieburth 1979; Waterbury et al. 1979). A few years later this discovery was extended to several lakes in Europe (Chang 1980; Cronberg and Weibull 1981; Hickel 1981; Craig 1985) and North America (Craig 1984; Caron et al. 1985b). However, it must be pointed out that numerous investigators had previously obtained indications of picoplankton-sized photosynthetic microorganisms without taxonomic characterization or identification of these organisms. Among these should be cited the "μ-algae" from subarctic Swedish lakes (Rodhe 1955) and the "little round green things" observed by Paerl (1977) in some New Zealand lakes, as well as size fractionation suggestions of photosynthesizing phytoplankters of size <3 μm in diverse oceanic (e.g. Saijo 1964; Reynolds 1973; Berman 1975) and lake regions (Votintsev et al. 1972; Costella et al. 1979; Munawar and Fahnenstiel 1982).

The term "picoplankton" was first proposed to denote planktonic single-celled microorganisms of particle size 0.2–2.0 μm diameter, and this term was originally intended to cover the bacterioplankton (Sieburth et al. 1978). However,

since the bacteriologists preferred to retain the latter term for the heterotrophic bacteria (e.g. Fuhrman et al. 1980; Ammerman et al. 1984; Hagström et al. 1984), it became customary to use the term picoplankton for *only* the photoautotrophic microorganisms of the said particle size, which included the chroococcoid cyanobacteria and the smallest known eucaryotic microalgae (Johnson and Sieburth 1982). Nevertheless, some investigators have continued to use the term picoplankton with respect to cellular particle size only and have scrupulously distinguished between the phototrophic and apochlorotic components of the picoplankton (Davis et al. 1985). Other investigators have qualified their use of the term picoplankton by adding epithets such as "autotrophic" or "photoautotrophic" or by inserting the prefix "phyto" to coin the term "picophytoplankton" (Li et al. 1983; Takahashi and Hori 1984; Smith et al. 1985).

According to Murphy and Haugen (1985), the term "ultraplankton" should have historical precedence over picoplankton, since it has been used for decades to denote plankters of size <10 μm in the largest dimension (Strickland 1960), and, from this point of view, the picoplankton itself may be considered as forming a particularly small-cell size component of the ultraplankton. These authors also pointed out that such a definition finds a place for eucaryotic ultraplankters, many of which are significantly larger than the picoplankter size range, lying in the size interval 2–5 μm. We believe that such ultraplankters should be placed within the category of "nanoplankton" recently redefined to contain organisms in the size range 2.0–20 μm (Sieburth et al. 1978). Such size separation became possible with the availability of improved filtration procedures using better quality filters with a range of pore sizes (Berman 1975; Hobbie et al. 1977; Caron 1983). Under these circumstances, we regard the term ultraplankton as too loose for precise particle size definition, and, while it may have served a useful function in earlier years (e.g. Reynolds 1973), it needs to be replaced by the lògical size boundaries defined by the sequence micro–nano–pico (Sieburth et al. 1978).

In this review, we propose to use the term picoplankton even for those cases (e.g. Murphy and Haugen 1985) where the term ultraplankton was previously preferred but with sufficient regard to the appropriate particle size (<3 μm). Since we will be entirely concerned with photoautotrophic or phytoplanktonic microorganisms, we will not repeat these epithets, except in special cases. In defining the scope of this review, we will consider all known scientific aspects of the oceanic and freshwater picoplankton hitherto studied. Among these aspects may be listed those belonging to the disciplines of limnology and oceanography, cytology and taxonomy, microbiology, phycology, physiology, and biochemistry, although not enough is yet known in some of these areas.

II. Summary of Picoplankton Occurrences Reported

Long before the discovery of cyanobacterial ubiquity in aquatic systems, there had appeared incidental reports on occurrences of picoplankton-sized cyanobacteria in some eutrophic ponds and lakes. Massive production of a chroococcoid cyanobacterium, with rod-like to ovoid-shaped cells (1 μm diameter, 1.5–3 μm length), was observed in a sewage oxidation pond near Leipzig (Germany) during late summer, causing a supersaturation of oxygen (Drews et al. 1961). Likewise, freshwater primary production by a similar organism of bacterial size was recorded by Loch Leven, Kinross, Scotland (Bailey-Watts et al. 1968). More recently, unidentified photosynthetic picoalgae were reported from a small meromictic lake in southeastern Ontario (Craig 1984). The picoplankton in this case was presumed to be cyanobacterial from its response to epifluorescence microscopy, while that found in Lake Tahoe (California–Nevada) was shown to be eucaryotic (Vincent 1982).

Many years before the identification and characterization of oceanic picoplankton, size fractionation studies of photosynthesizing phytoplankton had often suggested a considerable proportion of photosynthesis attributable to the smallest sized fraction (<3 μm or smaller, depending upon the pore size of filters used). Such indications were reported for surface waters collected from several stations in the Indian Ocean, including the South China Sea (Saijo 1964; Saijo and Takesue 1965). Similar indications were later reported for natural phytoplankton samples collected from the Barents Sea (Reynolds 1973), the Gulf of California (Berman 1975), the Oslofjord and subarctic area near Tromso, northern Norway (Throndsen 1978, 1979), the equatorial Atlantic Ocean off the coast of Guinea, West Africa (Herbland and Le Bouteiller 1981), the subtropical Pacific Ocean off Hawaii (Bienfang and Szyper 1981; Takahashi and Bienfang 1983), a swedish corner of the Baltic Sera (Larsson and Hagström 1982), the eastern Mediterranean Basin (Berman et al. 1984), the Scotia Sea (Reid 1983), and Antarctic surface waters off the coast between Cape Ann and Mawson (Probyn and Painting 1985).

Regarding the discovery of picoplankton identity in the oceans, phycoerythrin-rich chroococcoid cyanobacteria (size 0.9–1.3 × 1.8–2.2 μm) were observed in high concentrations at seven stations in the Arabian Sea in January 1977, at three Pacific stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor (Waterbury et al. 1979). At about the same time, similar cyanobacteria were reported from several locations in the Atlantic Ocean, including the Sargasso Sea, Rhode Island Shelf, Narragansett Bay, the Grenada Deep, and the Barbados Deep (Johnson and Sieburth 1979). Subsequently, abundant distributions of such cyanobacteria were reported from southern California coastal waters (Krempin and Sullivan 1981), several sites in the eastern tropical Pacific Ocean including the Costa Rica Dome (Li et al. 1983), locations in the Celtic Sea (Joint and Pomroy 1983) and the Baltic Sea including the Kattegat and Skagerrak (Schmaljohann 1984), the western North Pacific Ocean including the South China Sea (Takahashi and Hori 1984), Kaneohe Bay, Hawaii (Landry et al. 1984), the coastal waters off Japan including the Kuroshio and the Oyashio (Takahashi et al. 1985), the northern Foxe Basin of the eastern Canadian Arctic (Smith et al. 1985), the surface waters of the Mediterranean Sea (Fisher 1985), and the continental shelf waters of North and Northwest Australia (Hallegraeff and Jeffrey 1984). In some of these and other investigations, there was also evidence of eucaryotic picoplankters apart from the cyanobacteria (Manton and Parke 1960; Throndsen 1978, 1979; Johnson and Sieburth 1982; Joint and Pomroy 1983; Takahashi and Bienfang 1983; Takahashi and Hori 1984; Glover et al. 1985a, 1985b; Murphy and Haugen 1985). It appears from this summary that few marine regions remain yet uninvestigated for the occurrence and/or identification of picoplankton, among which may be mentioned

the Black and Red seas.

Regarding picoplankton occurrence in freshwaters, much remains to be done with respect to lakes in Asia, Africa, South America, and Australia, which appear from the literature to be virtually uninvestigated. The recent finding of cyanobacterial picoplankton in Lake Ontario (Caron et al. 1985b) and suggestions of similar picoalgae in Lake Superior (Munawar and Fahnenstiel 1982) implicate ubiquitous occurrence of picoplankton in the interconnected Great Lakes of North America. However, apart from mention of coccoid cyanobacteria in a Michigan Lake (Lehman and Sandgren 1982), size fractionation indications of similar picoalgae in three South Carolina reservoirs (Tison and Wilde 1981) and a small meromictic lake in Ontario (Craig 1984), the identification of *Synechococcus* in eight north-central Minnesota lakes (Fliermans and Schmidt 1977), that of *Synechocystis* spp. in freshwater enclosures in Cuba (Komarek 1985) and of eucaryotic picoalgae(?) in Lake Tahoe (Vincent 1982), and nutrient enrichment induced picoalgae in some coastal British Columbia and Yukon Territory lakes (Hardy et al. 1986; Shortreed and Stockner 1986), we get the impression that a considerable proportion of North American lakes remains uninvestigated for picoplankton. By contrast, more lakes have been investigated in Europe and picoalgae identified from Zurichsee, Switzerland (Chang 1980), seven eutrophic lakes in East Holstein, West Germany (Hickel 1981), Lake Trummen, southern Sweden (Cronberg and Weibull 1981), and 24 lakes from various regions of central and southern Sweden, the Aland archipelago, and northern Germany (Craig 1985). Apart from suggestions of picoalgae in Lake Baikal (Votintsev et al. 1972), we are not aware of comparable studies for picoplankton in the eastern European countries, largely on account of difficulty in access to their scientific literature.

III. Size Fractionation and Identification Procedures

Like the case of phytoplankton (Strickland and Parsons 1972), picoplankton particles have generally been separated from natural water samples by filtration first through a coarse-mesh (150–200 μm) net or screen (to remove the large zooplankton and detritus) followed by a second filtration through a membrane filter of smallest available pore size intended to retain all nano-, ultra- and picoplankton. This procedure was used for enumeration, characterization, and identification of size-measured picoplankton by epifluorescence microscopy (Waterbury et al. 1979; Johnson and Sieburth 1979; Krempin and Sullivan 1981; Caron et al. 1985a), but not size separation for purposes of assessing picoplankton contribution to total plankton photosynthesis or primary productivity. In the latter case, the natural water sample is first incubated with [^{14}C]bicarbonate and then filtered through a series of screens and membrane filters of diminishing pore size (e.g. 5,1, and 0.2 μm (Joint and Pomroy 1983); 8, 3, 0.8, and 0.2 μm (Murphy and Haugen 1985); 212, 40, 20, 8, 5, 3, and 1 μm (Takahashi and Bienfang 1983)) followed by standard scintillation counting of the ^{14}C fixed by each size fraction.

The performance and reliability of screens and filters for size separation of marine particulate material have been well investigated (Sheldon and Sutcliffe 1969; Sheldon 1972). These authors pointed out that there is a basic difference in function between a filter and a screen. Whereas a filter is normally used only to remove particles from a fluid, a screen is like a sieve expected to separate two size fractions quantitatively. They found that (1) all the filters investigated removed from suspension many particles very much smaller than the stated pore size of the filter, but membrane filters removed relatively more material than other types, and (2) the stated pore size of a filter is not a reliable indicator of its effectiveness for separating size fractions of marine particles. More detailed studies by Sheldon (1972) indicated that the average minimum sizes of particles retained by metal membranes (Flotronics) and perforated polycarbonate membranes (Nuclepore) were comparable with the stated pore sizes when relatively small seawater samples with moderate particle concentrations were filtered, but when large samples or high particle concentrations were filtered the average retention size was less than the stated pore size. Furthermore, all cellulose ester membranes (Millipore) retained particles much smaller than the stated pore size, even from small samples with low particle concentration, and glass-fiber filters showed similar retention characteristics. Regarding the degree of vacuum used for the filtration, it was concluded that this was unimportant when a filter is used only for retention, but low vacuum (10–20 cm Hg) is preferable when the filter is used for size separation. Since these findings and other technical advantages reported by Hobbie et al. (1977), it has become common practice to use Nuclepore filters of pore sizes in the range 3.0–0.2 μm for separating and investigating marine algal picoplankton (e.g. Berman 1975; Douglas 1984), although some reservations have been expressed about the influence of the filtration vacuum used (Li et al. 1983; Smith et al. 1985). Significant cell loss from rupture of some fragile nanoplankter species was noted from using the conventional suction–filtraton procedure for phytoplankton after incubation with [^{14}C]bicarbonate, and this cell breakage was traced to air exposure of the cells on filters between the initial filtration and the subsequent rinsing steps (Goldman and Dennett 1985). These authors recommended avoiding such air exposure of the filtered cells and replacing the rinsing step by acid soaking (or fuming) to eliminate the unfixed $^{14}CO_2$.

Murphy and Haugen (1985) pointed out that the separation of cells into size fractions by filtration is, at best, inexact, being influenced by cell shape and cell wall flexibility in addition to cell concentration and filtration vacuum. They noted that certain picoplankters passing through the 0.8-μm filter actually measured 1.0–2.0 μm in diameter. It appears that the ideal cell size sorting technique may lie in the application of flow cytometry, a general term for the rapid measurement of particles in a moving fluid where information is obtained through their optical properties (Yentsch et al. 1983). The design of a flow cytometer for analyzing fluorescence signals from individual phytoplankton cells was recently described by Olson et al. (1983). An application of this technique to shellfish feeding on a mixed algal diet has demonstrated its ability to distinguish algal particles quantitatively, even when they are of similar size, by the detection of their fluorescent photosynthetic pigments (Cucci et al. 1985). The method has been hitherto applied to only nanoplankton-sized particles but is expected to work equally well with particles of the size of bacteria and cyanobacterial picoplankton.

In the absence of flow cytometry, the recognition and characterization of cyanobacterial picoplankton was considerably facilitated by the application of epifluorescence microscopy. Such microscopy was first developed for the visualization and enumeration of heterotrophic bacteria and/or microprotozoa

TABLE 1. Summary of algal in vivo fluorescence spectral signatures reported in the literature.

Algal type	Photosynthetic pigments		Wavelength (nm)		Reference
	Principal	Accessory	Excitation[a]	Emission	
Cyanobacteria	Chlorophyll *a*	Phycocyanin	615	647	Caldwell 1977
	Chlorophyll *a*	Phycoerythrin	490	560–570	Yentsch and Yentsch 1979
Cryptomonads	Chlorophyll *a*	Phycoerythrin	490	560–570	Yentsch and Yentsch 1979
Chlorophyta	Chlorophyll *a*	Chlorophyll *b*	450	680	Yentsch and Yentsch 1979
	Chlorophyll *a*	Chlorophyll *b*	478	678	Caldwell 1977
Diatoms	Chlorophyll *a*	Fucoxanthin	525–530	680	Yentsch and Yentsch 1979
Dinoflagellates	Chlorophyll *a*	Peridinin	525–530	680	Yentsch and Yentsch 1979

[a]Some of the excitation wavelengths shown are not the maxima for the corresponding accessory pigments, but they have been chosen to provide maximum scope for differentiation from the other algal types.

with the help of specific metabolite-binding fluorochromes such as acridine orange (Hobbie et al. 1977; Daley 1979), fluorescein isothiocyanate (Fliermans and Schmidt 1975; Sherr and Sherr 1983), 4′,6-diamidino-2-phenylindole, commonly known as DAPI (Porter and Feig 1980), proflavine hemisulfate (Haas 1982), and primulin (Caron 1983). No fluorochrome was required for the autofluorescing algae, where advantage was taken of the autofluorescence spectral signatures (characteristic in vivo excitation and emission spectra) unique to the cyanobacteria (Caldwell 1977; Yentsch and Yentsch 1979). A glance at Table 1 shows that phycoerythrin-containing cyanobacterial picoplankters are particularly well differentiable from the other algae (excepting cryptomonads) by their autofluorescence from excitation at 450–500 nm producing a yellow to orange appearance under the epifluorescence microscope (Waterbury et al. 1979; Caron et al. 1985b). The likelihood of autofluorescence interference from phycoerythrin-rich cryptomonads remains a distinct possibility, however, and has been actually recorded for Chesapeake Bay estuarine samples (Exton et al. 1983). In most of the above cited field investigations, it was common practice to concentrate the picoplankton along with other phytoplankton by filtration of natural water samples on 0.2-μm Nuclepore filters, previously counterstained with Irgalan black in order to eliminate the autofluorescence native to these filters (Hobbie et al. 1977; Daley 1979). The filtered picoplankton was enumerated from the particle size and nature of its autofluorescence, the cyanobacterial component fluoresceing yellow to orange and the eucaryotic component fluoresceing red. According to Davis and Sieburth (1982), the presence of heterotrophs in the same water sample can be simultaneously determined by preparing two filtered aliquots, one unstained for enumerating autofluorescent phototrophs and the other stained with acridine orange for visualizing the heterotrophs including bacteria and microprotozoa. Other workers have pointed out that acridine orange suffers from the irksome drawback of not enabling counts of both phototrophs and heterotrophs on the same filtered sample, while the use of other fluorochromes overcomes this drawback and permits the determination of both trophic types of planktonic microorganisms on one filtered sample (Haas 1982; Fliermans and Schmidt 1975; Sherr and Sherr 1983; Caron 1983).

The above described autofluorescence technique for identifying phycoerythrin-rich cyanobacteria (PECB) is not adequate for the phycocyanin-rich cyanobacteria (PCCB), whose fluorescence spectral signature is quite different (see Table 1). It would appear from the excitation and emission wavelengths stated by Caldwell (1977) that the PCCB may not be visually distinguishable from the red autofluorescence emitted by all chlorophyll-containing algae. According to Murphy and Haugen (1985), the PCCB fluoresce brilliant red-orange with 560 nm excitation and pale-red or not at all with the 490–500 nm excitation normally used to identify the PECB; thus, the PCCB fluorescence would tend to be confused with the red autofluorescence of chlorophyll. In this connection, a radically different identification approach was announced by Campbell et al. (1983), who used an indirect immunofluorescence technique applicable to both PECB and PCCB as well as to different genera of the chroococcoid cyanobacteria. It must be pointed out, however, that this technique had already been developed by Fliermans and Schmidt (1977) as a tool for the autoecological study of a freshwater strain of *Synechococcus*. In principle, this approach consisted in preparing antisera directed against several cultured strains of PECB and PEEB acting as antigens, followed by testing the antisera for cross-reactions with a wide variety of cyanobacteria. Each antiserum was found highly specific for the cyanobacterial strain against which it had been produced, and the observed cross-reactions occurred between strains that were isolated from similar aquatic environments.

Owing to its ultraminute size, there is a general consensus that the ultimate identification of a picoalga requires its ultrastructural examination by transmission electron microscopy (TEM). In fact, such examination gave the first clues to the eucaryotic, in addition to the procaryotic, nature of marine picoplankton cells (Johnson and Sieburth 1982). The entire methodology required for satisfactory concentration and cytological preservation of nanoplankton cells as well as procedures leading to sample preparation for TEM were first described by Bisalputra et al. (1973). Although not tested on field samples, we believe that this procedure would be equally applicable to picoplankton, since it was successfully used for a cytophysiological study of a marine phycocyanin-rich, picoplankter-sized chroococcoid cyanobacterium in culture (Kalley et al. 1977). Whereas this method uses vacuum-controlled filtration for concentrating the plankton from water samples, other investigators have used centrifugation of the picoplankton fraction prior to TEM examination (e.g. Larsson et al. 1978; Johnson and Sieburth 1982). However, the cell fixation technique for the TEM examination has been generally comparable (e.g. Kursar et al. 1981). Several alternative phytoplankton concentration techniques have been reported in the literature, such as ultrafiltration with continuous stirring (Morris and Yentsch 1972) or filter–transfer–freeze (Hewes and Holm-Hansen 1983), but they have not been hitherto tested with picoalgae.

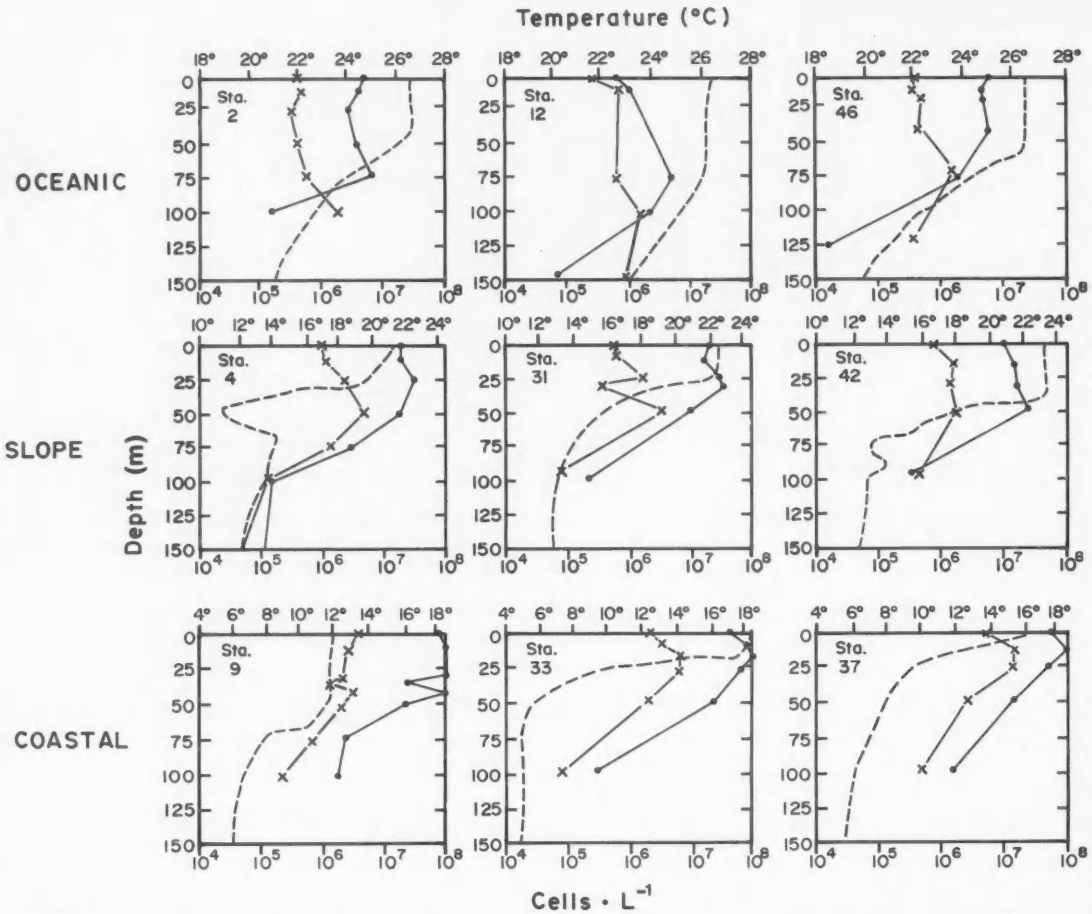

FIG. 1. Comparison of the vertical distributions of chroococcoid cyanobacteria (●) and ultraplankton-sized eucaryotes (×) in vertically stable water columns at nine stations in the North Atlantic Ocean. The broken line represents the temperature profile with depth. Note that a significant proportion of the eucaryote numbers shown may lie in the 2–5 μm diameter size range. (Reproduced with permission from Murphy and Haugen 1985)

IV. Component Organisms and Taxonomic Observations

Apart from enumeration by epifluorescence microscopy, a quantitative separation of the procaryotic and eucaryotic components of picoplankton has not yet been achieved. However, TEM sections of total filtered samples have given rough indications of the proportions of the two components (Johnson and Sieburth 1982; Joint and Pipe 1984; Takahashi and Hori 1984). In addition, enrichment cultures were initiated with water samples enriched with nutrients corresponding to established culture media, while single-cell isolations served as inocula for initiating unialgal cultures (e.g. Murphy and Haugen 1985). According to Johnson and Sieburth (1982), unicellular (eucaryotic) green algae developed in enriched cultures from every (coastal or offshore) station sampled on three cruises, while the red chroococcoid cyanobacteria grew only in enriched cultures from offshore stations.

Some comparisons (Fig. 1) of the vertical distributions of marine cyanobacteria and eucaryotes in stable water columns (both oceanic and coastal) showed (1) a general outnumbering (by an order of magnitude) of eucaryotes by cyanobacteria in surface waters and (2) a tendency for the numerical difference between the two types to diminish with depth until they virtually equalize at 75–100 m (Murphy and Haugen 1985). According to these investigators, total cyanobacterial numbers were highest (approximately 10^7–10^8 cells·L^{-1}) in coastal waters and lowest (10^6–10^7 cells·L^{-1}) in oceanic waters, at least with respect to the North Atlantic. Similar concentration levels were reported for the cyanobacteria observed in waters of the Arabian Sea and Central Pacific waters off the coast of Peru (Waterbury et al. 1979). However, according to these authors, the cyanobacterial concentration levels were consistently lower by an order of magnitude for the Slope water north of the Gulf Stream and Woods Hole Harbor water. The implied suggestion that cyanobacterial numbers decrease with increasing latitude was later explained as a water temperature effect (Murphy and Haugen 1985). This temperature effect on cyanobacterial densities was particularly evident from seasonal studies on Lake Ontario, where the cell densities ranged from

approximately 10^5 cells·L^{-1} in April (with temperatures of 0–5°C) to 10^8–10^9 cells·L^{-1} in August with a surface temperature of approximately 16°C (Caron et al. 1985b).

Whereas the chroococcoid cyanobacteria generally outnumber the eucaryotic picoalgae, an exceptional case of a *Chlorella* sp. outweighing cyanobacteria was reported for Kaneohe Bay, Hawaii (Landry et al. 1984). However, we wonder whether the said eucaryote was of picoplankton size, since no cell dimensions were stated. Nevertheless, it appears from a more extensive investigation (made during a cruise from the Gulf of Maine to the Sargasso Sea) that eucaryotes (of size <3 μm) can numerically dominate the picoplankton at the bottom of the euphotic zone (with 0.5% light level) where they could make a major contribution to the chlorophyll maximum (Glover et al. 1985b). In fact, these investigators noted that (1) surface (2 m) picoplankton assemblages were almost exclusively composed of phycoerythrin-rich cyanobacteria, with smaller size (0.2–0.8 μm) cyanobacteria predominating, (2) the proportion of larger size (0.8–3.0 μm) to smaller size cyanobacteria increased below the surface mixed layer, and the eucaryotic component often became important, and (3) at two Sargasso Sea stations, the greatest number of cyanobacteria were below the mixed layer at the 1% light level, while the maximum number of eucaryotic picoplankters occurred deeper still, at the 0.5% light level, coincident with the chlorophyll maximum. In another field investigation involving water masses in the Gulf of Maine, it was further revealed that (i) chroococcoid cyanobacteria played a more prominent role in phytoplankton communities at the least productive stations, (ii) ratios of in vivo fluorescence of phycoerythrin to chlorophyll *a* were always greater at the subsurface pigment maximum than at the surface, and (iii) cyanobacteria from the subsurface pigment maxima demonstrated greater phycoerythrin fluorescence intensity and photosynthetic rates than cyanobacteria from the surface (Glover et al. 1985a).

Procaryote Taxonomy

All the identified picoplanktonic cyanobacteria were assignable to the order Chroococcales reserved by taxonomists for unicellular species that divide by binary fission in one or more planes at right angles to one another (Stanier et al. 1971); hence the common usage of the epithet "chroococcoid" to characterize them. The genus *Synechococcus* figures most prominently among the taxonomic identifications made by the marine investigators cited in Section II including the discoverers of marine cyanobacterial picoplankton (Johnson and Sieburth 1979; Waterbury et al. 1979) and some of the most recent investigators (e.g. Guillard et al. 1985). Likewise, *Synechococcus* species have been commonly identified from lakes and ponds (Drews et al. 1961; Bailey-Watts et al. 1968; Chang 1980), but rare picoplanktonic species such as *Cyanodictyon reticulatum*, *C. imperfectum*, and *Cyanonephron styloides* have also been recorded (Hickel 1981, 1985; Cronberg and Weibull 1981).

The TEM studies of Johnson and Sieburth (1979) have indicated three types of marine *Synechococcus* cells as well as *Gloeocapsa*-like cells, while enrichment cultures produced some clones rich in phycoerythrin and others with phycocyanin. It is not known if the different types of *Synechococcus* cells reflect different species or various physiological states of the same species. Species identification in this genus has remained extremely problematical (Stainer et al. 1971; Rippka et al. 1979), while an ultrastructural study of a *Synechococcus* sp. (previously misnamed *Agmenellum quadruplicatum*) showed fine-structure polymorphism (five types of coccoid cells) from prolonged darkness exposure stress (Kalley et al. 1977). In the absence of species level identification, it is common practice to use clone or isolate labels (e.g. Guillard clone DC-2) assigned by the corresponding isolator or culture collection.

The first TEM study of a marine phycocyanin-rich *Synechococcus* (provisionally named *S. oceanica*) was due to Hall and Claus (1965). Corresponding examination of phycocyanin-rich freshwater *Synechococcus* species (*S. plancticus* by Drews et al. (1961) and *Anacystis nidulans* by Allen (1968a, 1968b) and Lawry and Jensen (1979)) showed much similarity in the fine structure of the cell wall, cytoplasm, and nucleoid to one another and also to *S. oceanica*. More recently, two marine isolates of *Synechococcus* (one phycocyanin rich and the other phycoerythrin rich) were found, on TEM examination, to possess spinae attached to an outer wall layer not found in previously described species (Perkins et al. 1981). Similar spinae, reminiscent of (but not identical to) bacterial spinae, were detected from TEM examination of another marine *Synechococcus* isolate (Sarokin and Carpenter 1981). It has been suggested that the presence of such spinae may prove to be taxonomically distinctive. Interestingly, another marine *Synechococcus* (Guillard clone DC-2), presumably lacking spinae but possessing an unusual cell wall sheath, was found to show additional unusual properties (such as a novel phycoerythrin and aberrant phycobilisomes) requiring its placement in a new taxonomic group yet unnamed (Kursar et al. 1981).

Probably the most dramatic finding, among marine *Synechococcus* strains isolated in culture from several locations in the Atlantic Ocean, was that of a novel type capable of "swimming" motility through liquids at speeds of about 25 μm·s^{-1} (Waterbury et al. 1985). Ultrastructural studies and other observations by these investigators have failed to reveal any flagella or other visible organelles of motility, indicating that such cyanobacteria may be propelled by a novel mechanism, since it must be remembered that hitherto cyanobacteria had only manifested "gliding" motility requiring contact with a solid surface (Castenholz 1982).

Long before the discovery of marine picoplankton, several phycocyanin-rich species of chroococcoid cyanobacteria had been isolated into culture from inshore marine environments which included estuarine mud, seashore sand, or seaweed material (Van Baalen 1962). Since these species were not obtained from pelagic water samples, there is doubt about them being components of picoplankton. However, at least for two of these species, their growth in stirred cultures (with aeration) was comparable with that of nanoplanktonic algae in not requiring settlement or attachment to solid substrata (Antia and Kalmakoff 1965). From these species, pure cultures of *Agmenellum quadruplicatum* (cell size 2 × 3 μm) and *Anacystis marina* (cell size 0.8 × 1.0 μm) have served particularly well as representatives of marine chroococcoid cyanobacteria for physiological and biochemical studies (Cheng and Antia 1970; Desai et al. 1972; Antia et al. 1975a), in the same manner that *A. nidulans* served as a freshwater representative (e.g. Peschek 1979; Vierling and Alberte 1980).

The problem of distribution of phycocyanin-rich and phycoerythrin-rich chroococcoid cyanobacteria in picoplankton has not yet been adequately addressed, although a beginning has been made by Campbell et al. (1983) using the immunofluorescence technique described above (Section III). Accord-

TABLE 2. Morphological criteria selected by Rippka et al. (1979) for taxonomic placement of chroococcoid cyanobacteria in the genera shown.

Genus	Thylakoids present (+) or absent (−)	Reproduction by binary fission cell division in (sheath present (+) or absent (−)): one plane	2 or 3 planes
Synechoccocus	+	−	
Synechocystis	+		−
Gloeothece	+	+	
Gloeocapsa	+		+
Gloeobacter	−	+	

ing to these authors, preliminary results for April to December 1982 in Great South Bay, New York, indicated that (1) *Synechocystis* populations are present only during spring and summer, (2) phycocyanin-containing *Synechococcus* strains are only a minor component in the spring and summer, and (3) phycoerythrin-containing *Synechococcus* populations become significant in summer and remain so until late autumn or winter. A similar study using antibodies prepared against the freshwater phycocyanin-rich *Synechococcus cedorum* indicated cross-reactive, morphologically similar cyanobacterial cells occurring only at certain depths in the water column of several lakes in northern Minnesota, most commonly at or near the surface and below the thermocline; the numbers of such cells ranged from 4×10^4 to 10×10^6 cells·L^{-1} (Fliermans and Schmidt 1977). Although the production of phycocyanin or phycoerythrin preponderance by a chroococcoid cyanobacterium would appear to be a useful taxonomic marker, this property has not been so used because of its alleged unreliability in the face of chromatic adaptation (Cohen-Bazire and Bryant 1982); this question will be further considered in Section V.

It was pointed out by Stanier et al. (1971) that the taxonomy of chroococcoid cyanobacteria was, at that time, in a state of chaotic confusion because of scientifically unsupportable identification practices used by the phycologists such as comparison with herbarium specimens without a requirement for live cultures. These authors undertook a complete taxonomic revision by procuring all available strains in culture and making comparisons along the lines used by bacteriologists. As a result, several previously used genera (*Agmenellum, Anacystis, Aphanocapsa, Aphanothece, Coccochloris, Chroococcus, Microcystis*) were banished as superfluous (Rippka et al. 1979). These authors formulated two principal groups of chroococcoid cyanobacteria according to their capacity for reproductive division in (i) one plane or (ii) two or three planes; the critical characteristics of the accepted genera placed in these groups are listed in Table 2. However, it was soon realized that the general *Synechococcus* and *Synechocystis* in this scheme showed considerable irregularity among their respective members; in particular, they were characterized by wide DNA base compositional spans and by clear-cut internal discontinuities in base composition (Herdman et al. 1979). It appeared inevitable that each of these genera would be eventually split up into several new genera as more information on their properties was gathered.

In fact, a later proposal by Rippka and Cohen-Bazire (1983) decided to (1) restrict the term *Synechococcus* to clones meeting the requirements of Table 2 but possessing DNA base composition only in the range 47−56 mol% G + C and a cell diameter of 1−3 μm, (2) transfer from *Synechococcus* to a new genus *Cyanobium* those clones characterized by a high mean DNA base composition (66−71 mol% G + C) and, at the same time, measuring 0.8−1.4 μm in cell diameter, (3) transfer from *Synechococcus* to another new genus *Cyanobacterium* those clones characterized by a cell diameter of about 2 μm and a particularly low mean DNA base composition (39−41 mol% G + C), and (4) transfer from *Synechococcus* to a third new genus *Cyanothece* those clones (originally designated *Aphanothece*) characterized by a faculty of anaerobic N_2-fixation (unlike aerobic N_2-fixation characteristic of *Gloeothece* clones) as well as unusually large cell diameter (4−6 μm) and a DNA base composition of 42 mol% G + C. The stated size ranges indicate that only *Cyanothece* members clearly exceed picoplankter size. We are not aware of similar taxonomic revision with respect to *Synechocystis*. Furthermore, we get the impression from the subsequent literature that the above proposed taxonomic revision for *Synechococcus* has not yet been accepted by others, with the notable exception of Reed and Stewart (1985).

Among the banished genera, we wish to consider specifically the cases of species, such as *Agmenellum quadruplicatum, Anacystis marina*, and *A. nidulans*, which have figured prominently in numerous previous physiological and biochemical investigations (see Section V). Although these species have been unequivocally assigned now to the *Synechococcus* complex with strain numbers rather than species designations (Rippka et al. 1979), we will continue to refer to them by their original names for the sake of convenience. Nevertheless, we should point out several irregularities in the literature in connection with these species. When the misidentification of *A. nidulans* was realized, its name was changed several times in the literature to either plain *Synechococcus* (e.g. Ihlenfeldt and Gibson 1975, 1977; Lawry and Jensen 1979) or *Synechococcus leopoliensis* (e.g. Rigby et al. 1980; Mierle 1985) or *Synechococcus* 6301 (McKie et al. 1981). Furthermore, it appears from other publications (e.g. Resch and Gibson 1983) that either of two strains of *A. nidulans* (UTEX 625 and R-2) may have been involved in some of these studies. In view of such confusion, we have ignored strain differences and synonyms and retained the original name *Anacystis nidulans* for the sake of consistency. In the case of *Agmenellum quadruplicatum*, we have followed the same policy with the two clones (PR-6 and BG-1) originally isolated by Van Baalen (1962), although PR-6 appears to have been mostly used in the investigations of interest to this review.

Because of the overall difficulty of unequivocal identification of cyanobacteria and the greater reliance placed on assigning numbers to new isolates, we are not surprised to note, in the literature, an attempt to devise a descriptive numeriClature system for these microorganisms (Griffiths 1984).

Eucaryote Taxonomy

CHLOROPHYCEAE

The most commonly reported eucaryote from marine picoplankton was said to be a *Chlorella*-like coccoid green alga (Johnson and Sieburth 1982; Joint and Pipe 1984; Takahashi and Hori 1984). In fact, the first-mentioned authors claim to have observed, with the help of TEM, three types of *Chlorella*-like cells, one of which was probably a species of *Nannochloris*

approximately 1 × 2 μm in size, containing a single nucleus, mitochondrion, starch-producing chloroplast with four thylakoids per lamella, and a multilayered cell wall surrounding the plasma membrane. The second type of *Chlorella*-like cell was generally similar to the first type but had a single-layered cell wall, while the third type displayed an aberrant morphology in which the cytoplasm had withdrawn from the cell wall but still showed a starch-containing chloroplast, nucleus, and mitochondrion. The aberrant nature of the third type suggested that it could be an artefact of improper cell fixation. Judging from the electron micrographs shown, we believe that the *Chlorella*-like cells observed by Takahashi and Hori (1984) may correspond to the first type of Johnson and Sieburth (1982). Likewise, we infer that the three types of *Chlorella*-like cells observed by Joint and Pipe (1984) could correspond to the three types described above, apart from differing in some structural detail.

Two clearly identified *Chlorella* species, of picoplankton size, have been reported in the botanical literature. The marine species *Chlorella nana* (mean diameter 2.25 ± 0.41 μm) was isolated in culture from the northern Adriatic Sea, about 8 km off Venice (Andreoli et al. 1978), and later from the waters of a coral reef in the vicinity of Townsville, Australia (Thinh and Griffiths 1985). These authors have extensively examined its ultrastructure and some aspects of its growth physiology. The freshwater *Chlorella minutissima* (1.8−3.0 μm cell diameter) was first described from small pools of polluted water near Prague, Czechoslovakia (Fott and Novakova 1969), and later isolated into culture from the stagnant inshore waters of Lake Pupuke, Auckland, New Zealand (Dempsey et al. 1980). In addition to its minute size, this species is characterized by a thin cell wall with an outer sporopollenin-containing layer, and it contains a pyrenoid said to be absent from *C. nana*.

The first report of marine *Nannochloris* species approximating picoplankton size was due to Butcher (1952), who described *N. maculata* and *N. atomus* both with cell diameter approximately 3 μm. Recently, several marine *Nannochloris* strains of picoplankton size (<3 μm diameter) were reported as isolates from Great South Bay, New York (Sarokin and Carpenter 1982), or from other locations (Turner and Gowen 1984). Interestingly, a careful comparative ultrastructural examination of five *Nannochloris* strains revealed cytological features indistinguishable from those known for the genus *Chlorella*, so that the validity of *Nannochloris* as a distinct genus has been questioned (Sarokin and Carpenter 1982). Such confusion might explain the assignment of *Nannochloris* of a picoplanktonic *Chlorella*-like cell by Johnson and Sieburth (1982).

Among other unicellular chlorophycean algae reported in the literature that might barely fit the size dimensions of picoplankton, we should mention *Stichococcus cylindricus* (cells 2 × 3−4 μm) and *S. bacillaris* (cells 2 × 3−4 μm), both of which appear to be freshwater organisms capable of tolerating high salinities (Butcher 1952; George 1957; Hayward 1974). It has been pointed out that members of the genus *Stichococcus* are difficult to distinguish from those of the genus *Klebsormidium* if only the cytological characters are used, but the distinction becomes more practical from the identification of their osmoregulatory organic solute pools (Brown and Hellebust 1980). Finally, mention should be made here of the small-celled green alga *Monoraphidium contortum* found as a shade-adapted occupant of Lake Tahoe, California−Nevada (Vincent 1982). With the reported cellular dimensions (1.5−2.5 μm wide × 10−15 μm long), we consider this alga to belong more truly to the category of nanoplankton or ultraplankton.

Prasinophyceae

In addition to the chroococcoid cyanobacteria and *Chlorella*/*Nannochloris* cells, the picoplankton of North Atlantic waters was said to contain a prasinophycean flagellate (of cell size 1 × 1.5 μm) and a scale-bearing nonflagellated prasinophyte of cell size 0.5 × 1.0 μm (Johnson and Sieburth 1982). The former, tentatively identified as *Micromonas pusilla*, was abundant in estuarine samples in summer, while the latter was the most ubiquitous eucaryote being found in 9 of 15 different locations on 15 of 20 sampling dates in water samples from Iceland to the Caribbean Sea. Furthermore, this latter (nonflagellated) prasinophyte is believed to be the smallest known phototrophic eucaryote and has not been hitherto described in the algal literature. Neither of the above described nor other prasinophytes were seen in the picoplankton from the Celtic Sea (Joint and Pipe 1984), while a third type of prasinophyte, said to be a small-size version of a *Pyramimonas* species, was reported in the subtropical picoplankton of the western North Pacific Ocean (Takahashi and Hori 1984).

Micromonas pusilla had been previously reported in the ultraplankton from Norwegian coastal waters (Throndsen and Heimdal 1976; Throndsen 1976, 1978, 1979) and also observed earlier in British coastal waters where it had been misidentified as the chrysophyte *Chromulina pusilla* (Butcher 1952). An extensive TEM examination established its taxonomic position as a member of the Prasinophyceae (Manton and Parke 1960). Another *Macromonas* species, previously identified as *Thalassomonas minima* and found in pools along the salt marshes of British rivers, possessed cells measuring 1.5−2.0 μm in diameter (Butcher 1959; Parke and Rayns 1964). We are not aware of other picoplankton-sized prasinophytes, apart from a newly identified flagellate *Dolichomastix lepidota*, measuring approximately 2.5 × 1.5 μm without the flagella, which was reported from surface waters in the new harbor at Cape Town, South Africa (Manton 1977).

Eustigmatophyceae

Although no species from this algal class has been hitherto reported in size-fractionated natural picoplankton from lakes or oceans, it is not unlikely that the minute coccoid forms could have been mistakenly placed in other algal groups on account of difficulty in their identification. Such difficulties were pointed out by Antia et al. (1975b) and confirmed by Turner and Gowen (1984). According to Hibberd (1981), the genus *Nannochloropsis* was designed to contain unicellular free-floating coccoid or cylindrical eustigmatophytes measuring less than 5 μm in maximum dimension. The type species *N. oculata*, isolated from brackish supralittoral rock pools (on the island of Cumbrae, Scotland) and confused at that time with *Nannochloris* (Droop 1955), was reported to contain globose or subspherical cells measuring 2−3 μm in diameter (Turner and Gowen 1984), which cell size barely meets the upper size limit of picoplankton.

Bacillariophyceae

There has been only one report on the finding of a diatom in natural size-fractionated marine picoplankton, and this diatom was not taxonomically identified (Takahashi and Hori 1984). However, too few picoplankton studies have been made in this regard, and there is no reason per se why diatoms should not

TABLE 3. Reported ranges of valve dimensions for some very small marine diatoms.

Diatom species	Valve or frustule dimensions (μm)[a]	Reference
Bellerochea polymorpha	2.1−3.4 × 2.2−3.6	Hargraves and Guillard 1974
Bellerochea spinifera	2.0−3.2 × 1.9−7.0	Hargraves and Guillard 1974
Chaetoceros filiforme	3.0−4.0 ×4.0−8.0	Hendey 1964
Cylindropyxis profunda	1.8−3.0	Hendey 1964
Fragilaria pinnata	2.0−4.4 × 2.8−5.5	Hargraves and Guillard 1974
Fragilaria rotundissima	2.0−2.6 × 2.0−5.5	Hargraves and Guillard 1974
Minidiscus trioculatus	2.0−5.0 × 3.0−5.5	Hasle 1974
Phaeodactylum tricornutum[b]	3.0 × 8.0	Lewin 1958
Skeletonema costatum[b]	3.10−10.0 × 3.0−30.0	Hasle 1973
Skeletonema menzelii	2.7 − 7.0	Guillard et al. 1974
Synedra fragilarioides	2.0−2.5 × 2.8−5.3	Hargraves and Guillard 1974
Thalassiosira oceanica	3.0 − 12.0	Hasle 1983
Thalassiosira profunda	1.9 − 2.8	Hasle 1974
Thalassiosira pseudonana[c]	2.5 − 4.5	Guillard and Ryther 1962
Thalassiosira pseudonana[c]	2.5 − 5.5	Hasle 1983

[a]Depending upon the valve shape, the given dimensions represent diameter (or width or transapical axis) × length (or pervalvar or apical axis).
[b]Ovate type cell; Oslo Fjord specimens.
[c]Cell from blooms in Great South Bay and Moriches Bay, New York.

be more prevalent. Our search of the diatom literature has revealed several marine species where the smallest valve diameter barely fits the upper limit of picoplankton size bracket, while the other dimensions are often significantly larger. In fact, a glance at Table 3 shows that these species generally meet well the size requirements for ultraplankton, but where both cellular diameter/width and length are reduced to the lower limits shown (e.g. *Bellerochea polymorpha, B. spinifera, Fragilaria rotundissima*), the corresponding diatoms might well size fractionate with the picoplankton.

CRYPTOPHYCEAE

Although no cryptomonads have been hitherto reported from natural size-fractionated picoplankton, we must point out that, if present, they may have been "missed" from the samples analyzed on account of their fragile nature. At any rate, we have located in the phycological literature two cryptomonads of minuscule size, one of which barely fits the picoplankton size bracket. This one is a marine species, *Hillea marina*, with cell dimensions of 2.0 × 2.5 μm, which species was reported isolated from shellfish tanks at Conway, North Wales (Butcher 1952). The smallest known freshwater cryptomonad, *Rhodomonas pygmaea*, was isolated from Clear Lake, California (Javornicky 1976), but its cell dimensions (2.8−5.1 × 4.4−6.7 μm) preclude it from the picoplankton size bracket.

Other algal classes

One or more unidentified species of chrysophytes of cell length approximately 0.6−2.0 μm were recorded in phytoplankton from subtropical Hawaiian waters (Takahashi and Bienfang 1983), while other studies, using TEM, in the western North Pacific Ocean reported the occurrence of an unidentified prymnesiophyte in the picophytoplankton (Takahashi and Hori 1984). Our search of the phycological literature has failed to turn up any chrysomonad of such small size. Among the smallest recorded marine prymnesiophytes may be considered *Trigonaspis minutissima* of cell size 2.0−3.6 μm diameter (Thomsen 1980), *Chrysochromulina minor* of cell size 2.5−5.0 μm diameter (Parke et al. 1955), and *Emiliania* (*Coccolithus*) *huxleyi* (Guillard's Sargasso Sea clone BT-6) of cell size approximately 2.0−3.0 μm diameter (Cheng and Antia 1970), all of which should belong strictly to the ultraplankton or nanoplankton size bracket.

There is mention of an unidentified dinoflagellate in the picophytoplankton of the subsurface chlorophyll maximum layer in subtropical and tropical oceanic waters (Takahashi and Hori 1984). New dinoflagellate species are presumbaly involved here, since we are not aware of picoplankton-sized dinoflagellates in the protistan literature, apart from symbiotic and parasitic forms.

V. Picoplankter Physiology and Biochemistry

Cyanobacterial Component

Pigments and photosynthesis

The major photosynthesis pigments of chroococcoid cyanobacteria reported by several investigators are listed in Table 4. Although shown as the major type, chlorophyll *a* is actually the only chlorophyll known from these microorganisms in culture (Stransky and Hager 1970). Among the major carotenoids generally identified, zeaxanthin is by far the most preponderant, ranging in proportions of 40−80% total carotenoid (Table 4). This observation prompted Guillard et al. (1985) to deploy zeaxanthin as a characteristic cyanobacterial marker of picoplankton from, at least, the marine environment. It should be noted that all the species in Table 4 are of marine origin, excepting *Anacystis nidulans* and *Gloeobacter violaceous*. Interestingly, the association of zeaxanthin with chlorophyllous picoplankton-sized particles had already been previously noted for near-surface water samples from the tropical Atlantic Ocean off West Africa and the Caribbean Sea off the west coast of Curaçao (Gieskes and Kraay 1983). These authors also noted the occurrence of an unknown chlorophyll *a* derivative as a major pigment accompanying chlorophyll *a*, carotene, and zeaxanthin. Such a chlorophyll derivative has not been hitherto observed from cultured cyanobacteria and re-

TABLE 4. Major photosynthesis pigments of chroococcoid cyanobacteria. PC = phycocyanin; PE = phycoerythrin; β-Car = β-carotene; Zeax = zeaxanthin; Calox = caloxanthin; Myxox = myxoxanthophyll; Oscillax = oscillaxanthin; +++ = judged preponderant in the absence of quantitative determination; nd = not known.

Cyanobacterial species[a]	Chlorophyll type	Phycobiliproteins	Carotenoids (% of total)				
			β-Car	Zeax	Calox	Myxox	Oscillax
Agmenellum quadruplicatum (1,11)	*a*	PC	26	40	—	18	—
Anacystis marina (4)	*a*	PC	nd	nd	nd	nd	nd
Anacystis nidulans (2,3,7)	*a*	PC	28	37	22	—	—
Gloeobacter violaceous (4,10,13)	*a*	PC+PE	+++	—	—	—	+++
Synechococcus elongatus (5,6,7,12)	*a*	PC	22	65	3	4	—
Synechococcus clone WH 7803[b] (8,9,12)	*a*	PE	18	81	—	—	—
Synechococcus clone WH 8018 (8)	*a*	PE	nd	nd	nd	nd	nd
Synechococcus clone WH 5701[c] (8,12)	*a*	PC	28	52	16	—	—
Synechococcus clone WH 6501 (8,12)	*a*	PE	+++	+++	—	—	—
Synechococcus clone WH 8007 (12)	*a*	nd	22	77	—	—	—
Synechoccus clone WH 7805 (8)	*a*	PE	nd	nd	nd	nd	nd

[a]References: 1, Antia and Cheng (1977); 2, Buchecker et al. (1976); 3, Evans and Allen (1973); 4, Rippka et al. (1974); 5, Stransky and Hager (1970); 6, Hertzberg et al. (1971); 7, Chapman (1973); 8, Alberte et al. (1984); 9, Kursar et al. (1981); 10, Guglielmi et al. (1981); 11, Gray et al. (1976); 12, Guillard et al. (1985); 13, Bryant et al. (1981).

[b]This clone has also been designated as "DC-2" (Alberte et al. 1984; Kursar et al. 1981).

[c]This clone answers the description of *S. bacillaris* Butcher (1952) and is considered identical with it (Guillard et al. 1985).

TABLE 5. Carotenoid proportions found in different membranes of chroococcoid cyanobacteria. Thy = thylakoid membrane; CW = cell wall; Cyp = cytoplasmic membrane; Omcw = outer membrane of cell wall (I and II = fractions I and II). References: 1, Murata et al. (1981); 2, Omata and Murata (1983); 3, Jürgens and Weckesser (1985); 4, Omata and Murata (1984).

Carotenoid	% total carotenoid							
	Anacystis nidulans				*Synechocystis* PCC 6714			
	Ref. 1		Ref. 2		Ref. 4		Ref. 3	
	Thy	CW	Thy	Cyp	Thy	Cyp	Omcw-I	Omcw-II
β-Carotene	37	1	53	3	43	4	13.0	8.1
Cryptoxanthin	1	0	2	1	2	1	—	—
Zeaxanthin	47	78	24	60	20	31	14.3	15.2
Caloxanthin	2	0	15	25	—	—	—	—
Nostoxanthin	12	19	6	11	—	—	—	—
Echinenone	—	—	—	—	11	1	12.1	13.9
Myxoxanthophyll	—	—	—	—	—	—	53.1	46.7
Unidentified	1	2	—	—	24	63	7.5	16.1

quires further investigation. Although not clearly related to the picoplankton fraction, unidentified chlorophyll *a* derivatives were also recorded for the tropical phytoplankton of shelf waters of north and northwest Australia (Hallegraeff and Jeffrey 1984).

Whereas the carotenoids are generally believed to be exclusively associated with the thylakoids, it was surprising to find a considerable proportion of them in the cytoplasmic membrane (Omata and Murata 1983), cell wall or cell envelope (Murata et al. 1981; Resch and Gibson 1983), and ultimately in the outermost (cell wall) membrane free from cytoplasmic or thylakoid membranes and peptidoglycan components (Jürgens and Weckesser 1985). Table 5 shows the carotenoid proportions reported by various investigators in the different membranes of the chroococcoid cyanobacteria examined. Such cell wall location of the carotenoids (preponderantly xanthophylls indicated by Table 5) might facilitate their much touted (and recently verified) role of providing photoprotection against harmful UV and visible radiation as well as O_2 supersaturation, and thereby increasing the photosynthetic performance of cyanobacterial populations at or near the water surface (Paerl 1984).

The phycobiliproteins of cyanobacteria are packed in special aggregated structures, termed phycobilisomes, which are themselves aligned in rows along the thylakoids (Evans and Allen 1973; Gantt 1975; Cohen-Bazire and Bryant 1982), excepting the rare organisms lacking thylakoids where the phycobilisomes lie adjacent to the inner surface of the cytoplasmic membrane (Rippka et al. 1974; Guglielmi et al. 1981). There is a general consensus that the phycobiliproteins function as antennae for light-energy trapping by chlorophyll in the spectral region 500–650 nm where light absorption by chlorophyll is poorest, and they are so organized within the phycobilisome that the energy from the trapped photon is transmitted stepwise via a series of phycobilins to chlorophyll *a* as illustrated in Fig. 2 drawn from Gantt (1975). This operation requires that (1) phycocyanin (PC) rich organisms must contain at least minor amounts of allophycocyanin (APC) in their photo-

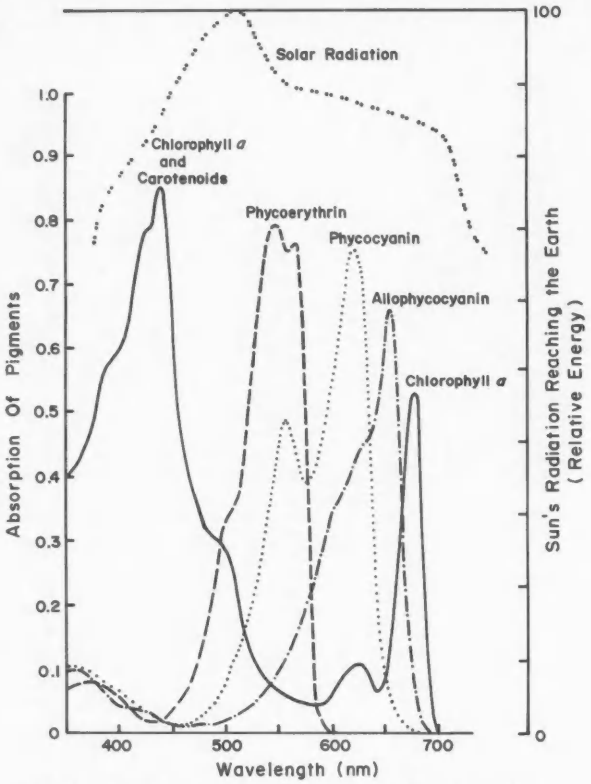

FIG. 2. Absorption spectra of phycobiliproteins from the phycobilisome (of phycoerythrin-rich microorganism) and their relation to chlorophyll absorption and solar radiation reaching the earth. The fluorescence peaks of these pigments closely overlap forming an integrated energy transfer system in the spectral region where chlorophyll absorption is poorest. (Reproduced with permission from Gantt 1975)

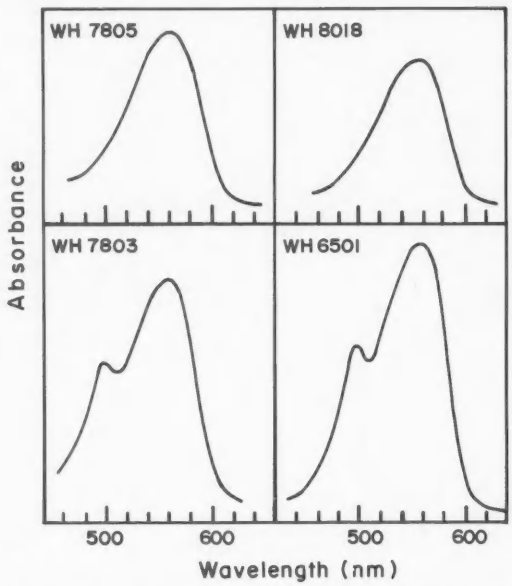

FIG. 3. Absorption spectra of purified novel phycoerythrins from four marine *Synechococcus* clones. The spectra indicate Type I phycoerythrins in clones WH 7803 and WH 6501 and Type II phycoerythrins in clones WH 7805 and WH 8018. The peak at 558 nm found in the phycoerythrin from all four clones is due to the presence of a phycoerythrobilin-like chromophore, and the peak at 500 nm in clones WH 7803 and WH 6501 is ascribed to the presence of phycourobilin chromophores. (Reproduced with permission from R. S. Alberte on behalf of Alberte et al. 1984)

synthetic unit (e.g. 96.5% PC + 3.5% APC in *Anacystis nidulans* grown under low light, according to Vierling and Alberte 1980) and (2) phycoerythrin (PE) rich organisms must additionally contain significant amounts of phycocyanin and allophycocyanin (e.g. 92% PE + 6% PC + 2% APC in *Synechococcus* clone WH 8018 according to Alberte et al. 1984). In some cyanobacteria (e.g. *A. nidulans*), an additional pigment, allophycocyanin B, was identified with spectral properties enabling it to function in energy transfer from allophycocyanin to chlorophyll *a* (Glazer and Bryant 1975).

After extensive purification, several phycobiliproteins showed unusual or novel properties. Two spectrally distinct phycocyanins (PC_{623} and PC_{637}) were initially isolated from the phycobilisomes of the phycocyanin-rich *Agmenellum quadruplicatum*, but these appeared on further examination to be different aggregated states of one in vivo phycocyanin (Gray et al. 1976). Similar aggregation differences appeared to be responsible for the fractionation of the biliproteins of *Gloeobacter violaceus* into phycoerythrin I and II, phycocyanin I and II, apart from allophycocyanin (Bryant et al. 1981), while the molecular ratios in the total phycobilisome were estimated at 1:3.9:2.9 for APC:PC:PE (Guglielmi et al. 1981). Interestingly, the former authors discovered a novel spectral feature in the phycoerythrin of *G. violaceus*, which was traced to the abnormal presence of phycourobilin (PUB) chromophores in addition to the normal phycoerythrobilin (PEB) chromophores in the proportion of 6 PEB per PUB. Similar novel (Type I) phycoerythrins were observed in 15 of 21 marine *Synechococcus* clones surveyed, where the ratio of PEB:PUB chromophores averaged 4.9:1.3 (Alberte et al. 1984). These investigators also observed another spectrally novel phycoerythrin (Type II) in the 6 remaining *Synechococcus* clones from the 21 marine clones surveyed, which pigment contained a phycoerythrobilin-like chromophore but no phycourobilin chromophore. The characteristic absorption spectra of the two novel types of marine *Synechococcus* phycoerythrins are illustrated in Fig. 3. An extreme case of Type I phycoerythrin was reported by Ong et al. (1984) from another clone (WH 8013) of marine *Synechococcus*, where the content of phycourobilin chromophore was the highest hitherto found; the ratio of PUB:PEB chromophores averaged 20.5:13, and the absorption spectrum, in contrast with the Type I spectra illustrated in Fig. 3, showed sharp peaks at 492 and 542 nm where the molar extinction coefficient at the former peak was about 2.4 times that at the latter peak.

Like the case of chlorophyll in marine phytoplankton (Strickland and Parsons 1972), sensitive fluorometric methods have been developed for the quantitation of phycoerythrin alone (Moreth and Yentsch 1970) or phycobilin mixtures from the presence of cyanobacteria and cryptomonads (Stewart and Farmer 1984) in seawater samples. Although these methods were designed for total phytoplankton, there is no reason per se why they should not apply to size-fractionated picoplankton.

Perhaps the most dramatic development in the methodology for the differentiation between the various phycobilin-containing *Synechococcus* clones and simultaneous measurement of the major phycobilins present in each clone was recently reported by Wood et al. (1985). After examining the in vivo spectral properties of nine clones of marine *Synechococcus* which included two clones lacking phycoerythrin, four clones rich in Type I phycoerythrin, and three clones rich in Type II phycoerythrin, these investigators developed a system using a combination of scanning spectroscopy, epifluorescence microscopy, and flow cytometry for in vivo differentiation and simultaneous estimation of the three pigment types as well as phycobilin-containing cryptomonads in one seawater sample. Furthermore, these investigators used the method for measuring in vivo phycobilin pigments at different stages of *Synechococcus* growth and under nutrient limitation, and established that the spectral signature of each clone remains the same under all growth conditions, implying genetic control of pigment biosynthesis subject to influences of light quality and quantity.

The biosynthesis of phycocyanobilin (the chromophore of phycocyanin) has been investigated in both the freshwater species *Anacystis nidulans* and the marine species *A. marina*, *Agmenellum quadruplicatum*, and *Coccochloris elabens* and was found to follow the glutamate pathway in all cases, where the biosynthesis proceeds from glutamate to δ-aminolevulinic acid and then to porphobilinogen, leading eventually to the phycobilin chromophore (Kipe-Nolt et al. 1978; Kipe-Nolt and Stevens 1980; Laycock and Wright 1981; McKie et al. 1981; Lewis et al. 1984). Phycobilin concentrations have been known to change with light intensity as well as with spectral quality. In the case of *A. nidulans*, high-light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) grown cells possessed a photosynthetic unit consisting of a core of approximately 157 chlorophyll *a* molecules per P700 associated with a light harvesting system of approximately 95 biliprotein chromophores, while low-light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$) grown cells had substantially more biliprotein chromophores (approximately 125) than the high-light cells but showed no significant difference in the numbers of chlorophyll *a* molecules per P700 (Vierling and Alberte 1980). These investigators also noted that low-light grown cells produced proportionately more phycocyanin relative to allophycocyanin than high-light cells (PC:APC was 27.5:6.3 in high light and 41:1.5 in low light). Using the same organism, other workers examined the effects of chromatic adaptation from cells grown in red or white light (Evans and Allen 1973). The chlorophyll to phycocyanin ratio in cells grown in white light was 2.35 times that in cells grown in red light. However, as a percentage of dry weight, the amount of phycocyanin was approximately the same in both types of cells, while the chlorophyll content in red-light cells was approximately one-third that of white-light cells.

There is a strong preponderance of phycoerythrin-rich chroococcoid cyanobacteria in tropical and temperate-zone oceanic and offshore marine regions during most of the year irrespective of the seasons (Waterbury et al. 1979; Glover et al. 1985b). According to Campbell et al. (1983), the phycocyanin-rich strains occur in minor concentrations only during spring and early summer. A survey of 21 *Synechococcus* clones cultured from various oceanic locations indicated not only that they were all phycoerythrin rich but that they all contained one or the other of the two novel phycoerythrins described above and spectrally illustrated in Fig. 3 (Alberte et al. 1984). According to Wood (1985), these phycoerythrin-rich *Synechococcus* strains have developed chromatic adaptation to the light quality and intensity available at the oceanic depth where they normally occur; however, this adaptation is not merely physiological but a consquence of evolutionary selection where the λ_{max} in vivo and the presence or absence of different phycobilins are under genetic control and independent of cell nutrient status. When the photosynthetic performance, under natural light, of two phycoerythrin-rich clones was compared with that of two phycoerythrin-lacking clones at depths of 15–60 m in the euphotic zone of clear ocean water, the phycoerythrin-rich clones showed considerable photosynthesis rates (6.8–8.4 $fg \cdot cell^{-1} \cdot h^{-1}$) at 60 m depth where the phycoerythrin-lacking clones showed little or no $^{14}CO_2$ uptake. Since such differences were not observed when artificial white light was used at different intensities simulating the natural light available at the water depths studied, Wood (1985) emphasized that it is the spectral quality of the natural light reaching the respective depths that is mainly responsible for the observed difference between the phycoerythrin-rich and phycoerythrin-lacking clones. This author further noted that the phycourobilin plus phycoerythrobilin chromophores are ideally suited to harvesting the blue-green component of natural light reaching those oceanic depths at the bottom of the photic zone, since the phycoerythrin-rich clones with such chromophores (see Fig. 3) showed superior photosynthesis at 60 m depth to that of the clones with phycoerythrobilin chromophores only.

Another notable feature of the marine *Synechococcus* clones, irrespective of their phycoerythrin content, is their tendency to saturate photosynthesis and growth rate at very low irradiances, while their photosynthesis is sensitive to oxygen concentration, suggesting a tendency for photorespiration (Morris and Glover 1981). Working with two oceanic clones (one phycoerythrin rich, the other phycocyanin rich), these authors observed (1) saturation irradiances for growth rate and photosynthesis at 45–55 and 110–130 $\mu E \cdot m^{-2} \cdot s^{-1}$, respectively and (2) maximum growth rate irradiances close to the growth rate saturation irradiances and (3) concluded that these characteristics were consistent with the existence of these organisms at or near the bottom of the euphotic zone. They also pointed out that primary productivity measurements made at normal (ambient) irradiances would tend to grossly underestimate the true contribution of cyanobacterial picoplankters on account of photosynthesis inhibition from these irradiances.

In another investigation, these authors thoroughly examined the previously noted photorespiration tendency of the same two *Synechococcus* clones (Glover and Morris 1981). By comparison with eucaryotic marine algae, the *Synechococcus* clones showed much greater potential for photorespiration judging from the following evidence: (i) a light-dependent O_2 inhibition of photosynthetic CO_2 assimilation at atmospheric O_2 concentrations, the degree of inhibition being dependent on the relative concentrations of dissolved O_2 and CO_2; (ii) actively photosynthesizing cells had high levels of ribulose-1,5-bisphosphate carboxylase relative to phosphoenolpyruvate carboxylase, and the former enzyme showed oxygenase activity three times greater than the carboxylase activity; (iii) cells photosynthesizing in approximately 20% O_2 showed significant ^{14}C-labelling of phosphoglycolate and glycolate and the percentage of total ^{14}C incorporated into these two compounds increased with increasing O_2 concentration. Interestingly, these authors noted further that the inhibitory effect of O_2 on photosynthesis did not appear to be solely due to photorespiration,

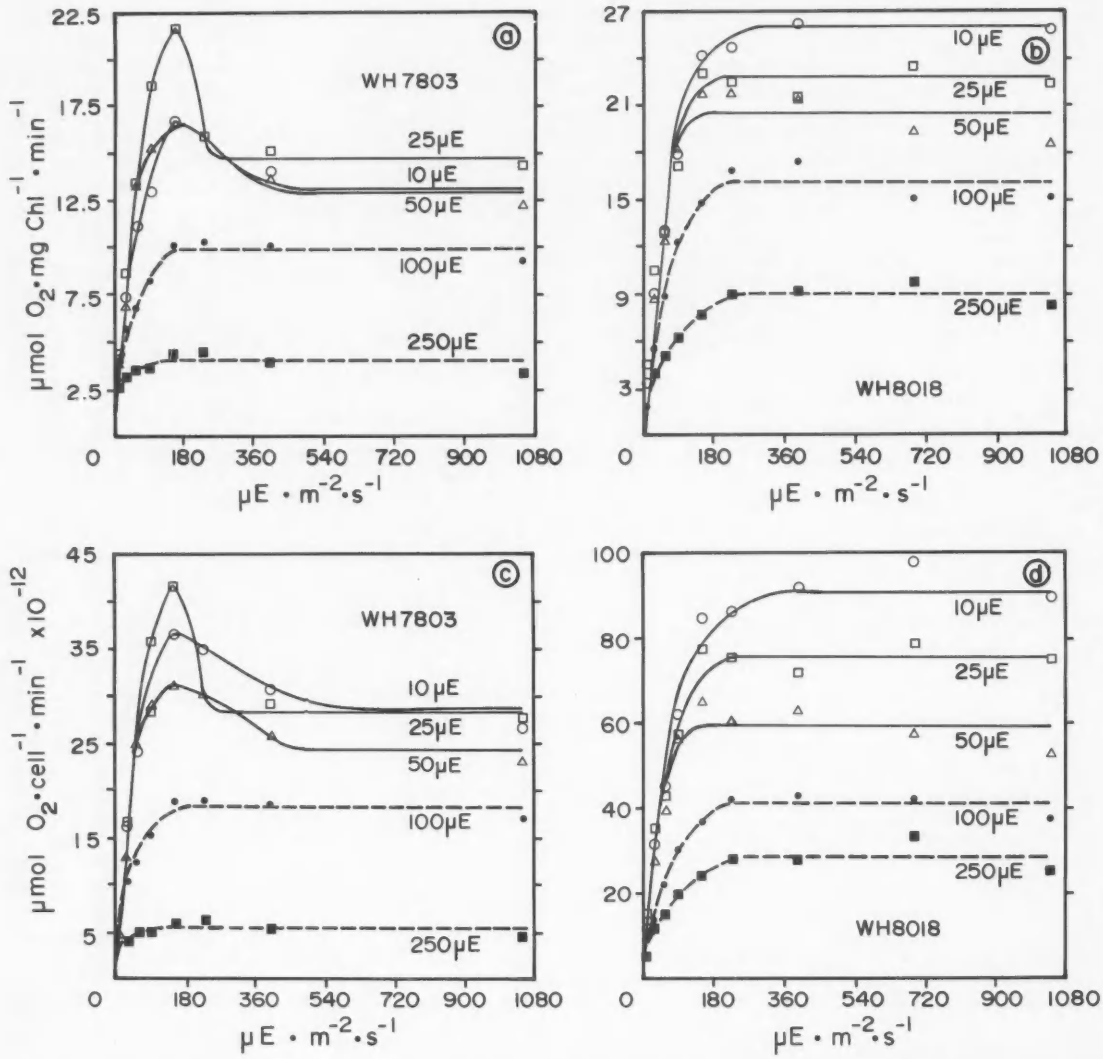

FIG. 4. Photosynthesis–irradiance relationships for *Synechococcus* clones WH 7803 (with Type I phycoerythrin) and WH 8018 (with Type II phycoerythrin) (a and b) normalized to chlorophyll *a* and (c and d) per cell. Values at each photon flux density are the mean of four replicates, and growth photon flux densities (10–250 $\mu E \cdot m^{-2} \cdot s^{-1}$, PAR) are indicated for each curve. (Reproduced with permission from Barlow and Alberte 1985)

since O_2 inhibition of photosynthetic O_2 evolution (1) was much greater than that of photosynthetic CO_2 assimilation and (2) declined only slightly with decreasing light intensities unlike the inhibition of CO_2 assimilation which declined rapidly with decreasing light intensity.

The photosynthesis sensitivity of two phycoerythrin-rich marine *Synechococcus* clones (WH 8018 and WH 7803, see Fig. 3) to elevated levels of light intensity was confirmed by Barlow and Alberte (1985), who noted that these clonal types optimize growth and photosynthesis at lower light intensities by increasing the numbers of photosynthetic units per cell and by decreasing photosynthetic unit size. These investigators observed, however, that while clone WH 7803 (with Type I phycoerythrin) was dramatically photoinhibited at high photon flux densities, clone WH 8018 (with Type II phycoerythrin) showed markedly less photoinhibition and its rates of photosynthesis were generally greater than those of clone WH 7803 (see Fig. 4). Despite the photosynthesis sensitivity of the latter clone (also known as DC-2), further studies by Wyman et al. (1985) disclosed that its production of phycoerythrin relative to phycocyanin depended not only on light intensity but also on the status of its nitrogen nutrition. These authors observed that at a moderately inhibitory irradiance (60 $\mu E \cdot m^{-2} \cdot s^{-1}$), this clone produced a PE:PC ratio of 12.5 in N (nitrate) limited chemostat cultures but a higher ratio of 21.6 in N-sufficient cultures. However, a high degree of energy loss through autofluorescence was detected upon excitation of the phycoerythrin in N-sufficient cells and evidence was obtained to indicate

inefficient energy coupling of the phycobilin to photosynthesis reaction-center chlorophyll. With further supporting evidence, these investigators concluded that phycoerythrin in clone DC-2 serves as a proteinaceous N reserve in addition to its normal function as a collector of quanta for photosynthesis.

Regarding the picoplankton as the size fraction passing through a 1-μm-pore filter, Platt et al. (1983a) determined several photosynthesis parameters for size-fractionated natural plankton assemblages from the chlorophyll maximum on the mid-Atlantic ridge around latitude 36°N. They found considerable differences in these parameters between the picoplankton fraction and the phytoplankton of size >1 μm; the assimilation number (P_m^B) and the initial slope (α) of the photosynthesis–irradiance curve were higher for the picoplankton fraction, while the adaptation parameter ($I_k \equiv P_m^B/\alpha$), the optimal irradiance (I_m), and the photoinhibition parameter (I_b) were lower. In attempting to explain the significance of these observations, Platt et al. (1983a) pointed out that (1) the elevated rate parameters (P_m^B and α) for picoplankton are consistent with the general tendency for rate variables to increase with decreasing cell size and (2) the elevated α for picoplankton could also be a consequence of more efficient absorption of light at smaller cell size.

Another field investigation (Glover et al. 1985a), comparing the photosynthetic characteristics of cyanobacteria-dominated picoplankton (<3 μm, >0.2 μm) with those of >3 μm phytoplankton, noted that the former differed from the latter in that (i) they utilized blue and in particular green light more efficiently for photosynthesis, but the pattern of photosynthetic carbon fixation into various "end products" was not affected by light quality for both >3 μm and picoplankton fractions, (ii) they required lower light intensities to saturate photosynthesis, (iii) the lower the in situ light level at the pigment maximum, the greater the contribution by picoplankton to total primary production, and (iv) the biochemical pattern of photosynthetic CO_2-fixation into "end products" did not vary significantly with decreasing light intensity for picoplankton, unlike the case of phytoplankton >3 μm where decreasing light intensity produced a relative increase in the proportion of carbon incorporated into polysaccharide, nucleic acid, and protein with a corresponding decrease in the carbon incorporated into lipid and small molecular weight metabolites (Fig. 5).

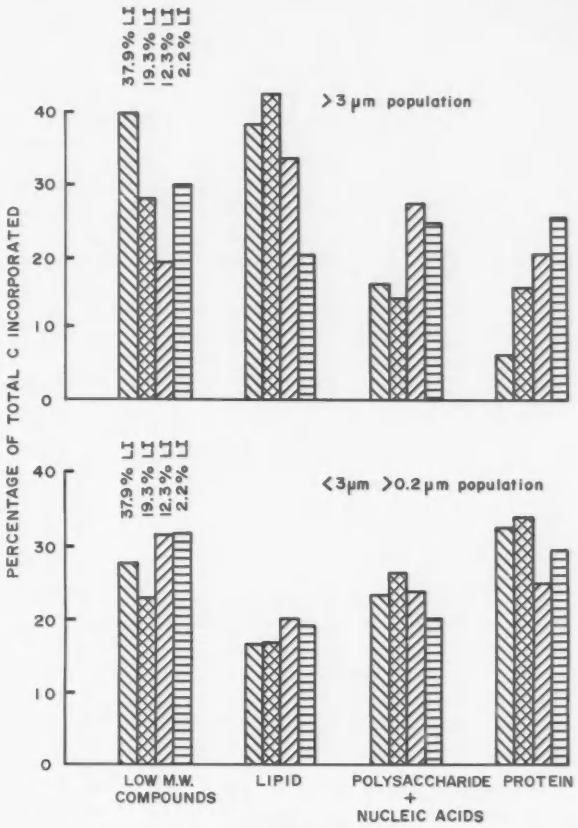

FIG. 5. Pattern of photosynthetic CO_2 fixation into various "end products" by <3 μm (picoplankton) and >3 μm phytoplankton fractions incubated at 37.9% (▨), 19.3% (▩), 12.3% (▧), and 2.2% (▤) of incident irradiance. Populations from the subsurface pigment maximum (18 m), at a station in the Gulf of Maine, were incubated in natural-light incubators cooled with surface seawater. Light intensity was reduced by neutral-density screens. (Reproduced with permission from H. E. Glover on behalf of Glover et al. 1985a)

Nutrient uptake and utilization

Nitrogen — Marine picoplankton (presumably cyanobacteria dominated) has been reported as capable of utilizing nitrate, ammonium, or urea as sole N source for growth, but significant preference for uptake and assimilation of the one or the other N source has been manifested in different bodies of water if not at each investigated site. In general, the <1 μm picoplankton fraction of the phytoplankton community in the southern Benguela upwelling system (off the southwest Atlantic coast of South Africa) as well as that in Antarctic surface waters (off the coast between Cape Ann and Mawson) showed a relative preference for reduced N in the form of ammonium or urea (Probyn 1985; Probyn and Painting 1985). In the former case, picoplankton was more active (27% of total phytoplankton) in oceanic N uptake relative to that (10%) in coastal waters; likewise, picoplankton turnover rates of particulate N were faster in oceanic than shelf or inshore waters (Probyn 1985). In the latter case, picoplankton accounted for about 90% N uptake of total phytoplankton in oceanic stations and 50% N uptake in offshore stations close to the ice edge, while all size fractions clearly showed discrimination against nitrate in spite of its domination (96%) of the nitrogenous nutrient pool, with ammonium being the most favored supply source (Probyn and Painting 1985).

A culture study evaluating the suitability of certain inorganic and organic N sources for phototrophic growth of marine microalgae from several taxonomic classes indicated that all the phycocyanin-producing cyanobacteria examined (viz. *Agmenellum quadruplicatum*, *Anacystis marina*, and *Synechococcus* clone PCC 7335) grew efficiently and equally well on nitrate, nitrite, ammonium, and urea but poorly or not at all on glycine, glucosamine, and hypoxanthine (Antia et al. 1975a). A more detailed study testing the utilization of numerous organic nitrogenous compounds showed *A. quadruplicatum* capable of growth on several amino acids and purines including glycine and hypoxanthine (Kapp et al. 1975). Since the same strain of *A. quadruplicatum* was used by the two groups of investigators, the observed discrepancy between them regarding glycine utilization was systematically examined by Bonin et al. (1982), who studied the influence of a

range of temperatures and irradiances on growth in the presence of glycine, with nitrate serving as control. This study concluded that (1) whereas nitrate supported good growth at all temperatures from 17 to 40°C, glycine gave significant growth only from 29 to 40°C, suggesting that the cyanobacterium is eurythermal with respect to nitrate but stenothermal with respect to glycine, (2) a minimum irradiance of 7.6 $\mu E \cdot m^{-2} \cdot s^{-1}$ as well as prolonged periods of adaptation are required for successful growth on glycine, and (3) the overall growth properties characterized the cyanobacterium as an estuarine warm-water organism. In another investigation (Nielson and Larsson 1980), *Synechococcus* clone PCC 6301 was found to utilize nitrate, ammonia, urate, and glutamine as N sources, while *Synechocystis* clone PCC 6714 utilized nitrate, urea, arginine, asparagine, and glutamine but not ammonia and urate; interestingly, glycine was not utilized by either cline under the test conditions used.

Nitrogen-deficient, phycocyanin-rich cyanobacteria have long been known to change color from blue-green to light-brown but the biochemical basis of "nitrogen chlorosis" remained undetermined until the systematic examination of the freshwater species *Anacystis nidulans* (Allen and Smith 1969) and the marine species *Agmenellum quadruplicatum* (Antia and Cheng 1977). In both cases, continuous photosynthesis with N starvation caused first the disappearance of phycocyanin followed by the gradual diminution of chlorophyll, leaving behind a preponderance of carotenoid. Since the addition of nitrate to a chlorotic culture resulted in the rapid restoration of normal pigmentation, it appeared that these cyanobacteria were using the phycocyanin as a N reserve under conditions of severe N deprivation and, in this respect, we regard the phycocyanin serving a similar additional function as phycoerythrin (discussed above) in *Synechococcus* clone DC-2 (Wyman et al. 1985).

The major pathway of inorganic N assimilation by chroococcoid cyanobacteria is believed to be similar to that known from eucaryotic microalgae, involving the sequence nitrate →nitrite→ammonium→glutamine→glutamic acid (Syrett 1981). Indirect evidence for this pathway has been obtained for *Anacystis nidulans* by adding specific, potent inhibitors of the key enzyme glutamine synthetase to nitrate-grown cells and noting the excretion and accumulation of ammonia in the culture medium. Micromolar amounts of L-methionine-S-sulfoximine (Ramos et al. 1982) or phosphinothricin (Lea et al. 1984) served as the specific inhibitor of glutamine synthetase. Such tests have not yet been reported for the phycoerythrin-rich marine *Synechococcus* clones discussed above. This is also true of the principal urea-utilizing enzyme, which has been shown to be urease in the phycocyanin-rich *Synechococcus lividus* (Berns et al. 1966) but has not yet been examined in any other member of the chroococcoid cyanobacteria.

Phosphorus — The investigation of P dynamics in a Michigan lake revealed that, during summer, the chroococcoid cyanobacteria possess extraordinary uptake capabilities for inorganic orthophosphate (P_i) serving as nutrient (Lehman and Sandgren 1982). From the moment of P_i enrichment, the cells were found to double their P content in 2 min or less but sustained such high uptake rates for only a few minutes. These authors concluded that, with equal initial access to the nutrient, the cyanobacteria were more successful than coexisting eucaryotes in converting the nutrient into new cell biomass. The above noted extraordinary P_i uptake capabilities of picoplankton-sized cyanobacteria may be accounted for by their extraordinary small cell size in accordance with the relationship between phytoplankton cell size and P_i uptake rate studied by Friebele et al. (1978), who found that smaller cells have a faster uptake rate per cubic micrometre of biomass than larger cells and inferred that smaller cells have a competitive advantage from possessing faster nutrient uptake rates. This competitive advantage of smaller cells was further confirmed by a more detailed investigation of cell size dependent P_i uptake kinetics including effects from cell nutrient quotas and application of Banse's (1976) "allometric" equation, although the picoplankton size bracket was overlooked in this case (Smith and Kalff 1982). It should be pointed out, however, that the competitive advantage conferred by small size on P_i uptake rate might disappear with increasing P_i concentration, since Lean and White (1983) reported predominant uptake by larger size phytoplankton when the exogenous P_i concentration exceeded 2 $\mu g\ P \cdot L^{-1}$.

Whereas the above-described investigation by Lehman and Sandgren (1982) reported a short-lived (few minutes) sustainability of the high uptake rate achieved from the moment of P_i enrichment, P_i perturbation studies on *Synechococcus*-dominated phytoplankton cultures, obtained from Kennedy Lake, Vancouver Island, B.C., showed little or no reduction of the uptake rate per cell in response to increasing cellular P over a period of at least 100 min (Suttle and Harrison 1986). This pattern of a sustainable high uptake rate per cell, while cell quota is rapidly increasing, is expected to confer considerable competitive advantage in lake areas where ephemeral pulses of elevated nutrient concentration exist. Evidence for such nutrient pulses has been reported for oceanic regions (Holligan et al. 1985), but not yet for any body of freshwater.

In the case of *Anacystis nidulans*, the cellular P level fell to about one seventh the normal content from prolonged starvation, but even then the cells were viable and continued to divide slowly (Batterton and Van Baalen 1968). Furthermore, these investigators noted that (1) P-depleted cells showed a rapid *dark* P_i uptake to restore cellular P content to a threshold subnormal value, after which light was required to bring cells to normal P content and normal growth rate, (2) coincident with the rapid *dark* P_i uptake, synthesis of ATP began and soared far above the normal cellular values, and (3) the rate of ATP formation was not influenced by the presence of light but was blocked by anaerobic conditions or several classical inhibitors of the electron transport chain.

Another investigation of phosphate uptake by *A. nidulans* revealed a strong influence of metallic cations, where all cations tested (excepting Mg^{2+} and Zn^{2+}) stimulated phosphate uptake (Rigby et al. 1980). These authors noted that Ca^{2+} exerted the most pronounced uptake enhancement by strongly diminishing the half-saturation concentration for phosphate uptake apparently by acting upon the metabolic processes of phosphate transport into the cell; at the same time, phosphate had no effect on Ca^{2+} fluxes across the cell surface. When the kinetics of phosphate transport by the same cyanobacterium were further investigated, deviations from Michaelis–Menten kinetics were observed at low phosphate concentrations, which deviations were attributed to diffusion limitation of phosphate transport (Mierle 1985).

An interesting connection between phosphate uptake (followed by intracellular deposition as polyphosphate) and sulfate-S deficiency was discovered for *A. nidulans* by Lawry and Jensen (1979), who noted that P uptake and polyphosphate

accumulation were greatest when the level of exogenous S was reduced to zero. A further investigation showed that such polyphosphate accumulation can serve as P source for growth when the S source is restored and exogenous P_i is depleted (Lawry 1976). Such catabolism of polyphosphate probably proceeds by the enzymatic pathways reviewed by Harold (1966) but has not yet been investigated with respect to chroococcoid cyanobacteria. The lack of P_i concomitant with sulfate-S provision also produced changes in the intracellular structural components and cell size of *A. nidulans* (Jensen and Rachlin 1984). It is interesting to note that polyphosphate-deficient mutants of *A. nidulans* have been prepared by the action of chemical mutagens and that these mutants show (1) absence of polyphosphate granules, (2) little growth in the absence of exogenous P_i, (3) altered polyphosphate kinase levels, and (4) severely retarded incorporation of $^{32}P_i$ (Vaillancourt et al. 1978).

As known from investigations on eucaryotic microalgae (see review by Cembella et al. 1984), *A. nidulans* also showed production of considerable alkaline phosphatase activity after the onset of phosphate-limited growth when the phosphate concentration in the growth medium fell to a value immeasurable by the assay method used (Ihlenfeldt and Gibson 1975). According to these authors, such phosphatase activity disappeared from addition of phosphate to the P-limited cultures, while its presence enabled growth of the cyanobacterium on several organic phosphate esters tested. Although not proven, it is not unlikely that the alkaline phosphatase may be located in the periplasmic space corresponding to layer three of the four-layer cell wall, as was histochemically established for *Plectonema boryanum* (Doonan and Jensen 1977).

In the case of marine chroococcoid cyanobacteria, a *Synechococcus* strain showed no phosphatase production nor ability to grow on organic phosphate ester when starved for P and exposed to glucose-6-phosphate (Kuenzler 1965). Interestingly, the same *Synechococcus* strain was found to excrete a considerable proportion of unidentified organic P when growing on P_i, which excretion was (i) light dependent but little affected by variation in light intensity and (ii) optimal at low salinity (2−5‰ but decreased rapidly with salinity increase beyond 10‰ (Kuenzler 1970).

Storage products and osmoregulatory metabolites

Among the generally occurring cyanobacterial inclusions reviewed by Allen (1984) may be listed several metabolite storage bodies, such as (1) *carboxysomes* (polyhedral bodies) for storage of the initial CO_2-fixation enzyme, ribulose-1,5-bisphosphate carboxylase, (2) *cyanophycin* (polypeptide) granules for storage of reduced nitrogen reserves, in addition to a similar secondary function served by the phycobilisomes, (3) *polyphosphate* (volutin) granules for storage of phosphorus reserves, (4) *polyglucose* bodies (α-granules) for carbohydrate storage in the form of glycogen, and (5) *poly-β-hydroxybutyrate* granules for storage of lipid reserves. The literature for these storage bodies has been well reviewed by Allen (1984), who has pointed out that (i) they generally become manifest from accumulation under specific starvation conditions and (ii) some of them may not be universally present in all types of cyanobacteria. A survey of several types of cyanobacteria for the occurrence of cyanophycin and polyphosphate bodies indicated that the *Synechococcus* strains examined produced the latter but not the former under all the nutrient deficiency conditions tested, while *Gloeocapsa alpicola* produced both (Lawry and Simon 1982).

Reed and Stewart (1985) examined the intracellular concentrations of low molecular weight carbohydrates and quaternary ammonium compounds present in 26 axenic isolates of chroococcoid cyanobacteria (mostly *Synechococcus* and *Synechocystis* strains) exposed to a range of external salinity ranging from freshwater up to 30‰ seawater and found that, in all cases, a single carbohydrate, either sucrose or 2-*O*-α-D-glucopyranosylglycerol (also known as glucosylglycerol), was identified as the principal organic osmoticum showing major variation in response to the external salt concentration, while quaternary ammonium compounds (such as glycine betaine) occurred in osmotically insignificant amounts. Whereas Mackay et al. (1983) considered the production of glucosylglycerol as unique to "marine" strains of cyanobacteria, Reed and Stewart (1985) pointed out that this viewpoint is fallacious because (1) glucosylglycerol-accumulating strains grew over the widest salinity range (up to 20−25‰ seawater), whether isolated from saline or nonsaline habitats, (2) sucrose-accumulating strains were more stenohaline and showed no sustained growth in hypersaline media (>100% seawater), and (3) the data suggested that the upper salinity limit for growth may be linked to the type of organic solute accumulation rather than habitat of isolation, with the glucosylglycerol-accumulating isolates having a greater potential for growth in salt-stressed conditions than the sucrose accumulators. A rather unexpected aspect of cyanobacterial low molecular weight carbohydrate (LMWC) accumulation was revealed when the combined effects of salinity and temperature were examined (Warr et al. 1985). According to these investigators, four glucosylglycerol-accumulating *Synechocystis* isolates also produced osmotically significant amounts (up to 50% total LMWC) of sucrose when grown in 100% seawater medium at 35°C but 95% glucosylglycerol at 20°C in the same medium. In contrast, these investigators also found three *Synechococcus* isolates that produced only sucrose at all temperatures and salinities tested.

Heterotrophic tendencies

There have been several reports of the heterotrophic potential of cyanobacteria in general where the chroococcoid members have been included, the best example being a recent review by Smith (1982). However, a few specific cases deserve mention here. Whereas chemoheterotrophy is rare, 9 of 38 chroococcoid cyanobacterial clones examined gave evidence of facultative photoheterotrophic growth on either of glucose, fructose, sucrose, or glycerol tested as carbon source (Rippka et al. 1979). The clones so tested included mostly freshwater and phycocyanin-rich organisms, and we wonder whether any of the oceanic phycoerythrin-rich *Synechococcus* clones (discussed in previous sections) have yet been tested for heterotrophic tendencies. Both *Agmenellum quadruplicatum* and *Anacystis marina* showed such tendencies (requiring light) with respect to glycerol as substrate (Cheng and Antia 1970). The glycerol-supported photoheterotrophy of *A. quadruplicatum* was confirmed by Ingram et al. (1973), who demonstrated not only efficient conversion of glycerol-carbon into cellular carbon but also the involvement of both Photosystems I and II (of photosynthesis) during the photoheterotrophic growth. Interestingly, *A. quadruplicatum* also grew heterotrophically in dim light with glucose as major source of carbon and possibly energy (Van Baalen et al. 1971). According to these authors, the dim-light conditions (i) did not support autotrophic growth

and (ii) induced greater contribution from glucose to cell constituents (especially amino acids) than high-light conditions. Regarding the mechanism of heterotrophic utilization of glucose, an excellent study by Pelroy et al. (1972) has pointed out that (1) a substantial fraction of the glucose consumed is assimilated and stored as glycogen, (2) the oxidation of glucose proceeds through the pentose phosphate pathway, whose first enzyme, glucose-6-phosphate dehydrogenase, is partly inducible and regulated by the intracellular concentration of a Calvin cycle intermediate, ribulose-1,5-bisphosphate, which acts as a specific allosteric inhibitor of this enzyme, and (3) as a consequence, the rate of glucose oxidation is greatly reduced by illumination, an effect reversed by the presence of DCMU known to inhibit Photosystem II. The overall results have led to a general hypothesis concerning the primary pathways of carbon metabolism in cyanobacteria, recently updated by Smith (1983).

Apart from the sugars, acetate has been shown to be taken up by *Anacystis nidulans* and *Aphanocapsa* clones only in the presence of light and assimilated largely into acetyl CoA followed by incorporation mainly into fatty acids and to a lesser extent into protein, while the rest of the intracellular carbon appeared to be derived from CO_2-fixation (Hoare et al. 1967; Ihlenfeldt and Gibson 1977).

Anaerobic (anoxygenic) tendencies

A faculty for growth by anoxygenic photosynthesis has been recorded for *Anacystis nidulans*, where oxidation of sulphide to elemental sulfur replaces the normal oxidation of water to O_2 (Pearson and Tomlinson 1975). According to Peschek (1978), thiosulfate or hydrogen can replace sulfide as electron donor for the anoxygenic photosynthesis, and, in all cases, evidence was obtained for the involvement of only Photosystem I. Subsequently, evidence was obtained for two functionally different hydrogenases in *A. nidulans*, one of which appeared to function in the anoxygenic photosynthesis system, while the other showed properties of an aerobic enzyme presumably functioning in respiratory utilization of H_2 by the oxyhydrogen reaction (Peschek 1979). Much of the literature on the hydrogen metabolism and related N_2-fixation of cyanobacteria has been excellently reviewed by Lambert and Smith (1981) and Bothe (1982) and will not be discussed further.

Nitrogen fixation

When first reported (Wyatt and Silvey 1969), N_2-fixation by a chroococcoid cyanobacterium (*Gloeocapsa* sp.) was greeted with much scepticism, since this finding violated the then prevalent "heterocyst privilege" dogma. However, this observation was confirmed by Rippka et al. (1971), who in a later investigation (Rippka et al. 1979) redesignated the organism as *Gloeothece* and also found four additional clones capable of fixing N_2 aerobically and the same number of these clones producing an abnormal phycoerythrin. These investigators also found three clones of *Synechococcus* (two phycoerythrin rich and one phycocyanin rich) able to fix N_2 under anaerobic conditions. Another case of aerobic N_2-fixation was reported by Singh (1973) for a strain of *Aphanothece*. It appears from the cited literature that virtually all the N_2-fixers originated from freshwater or brackish water sources, while the oceanic *Synechococcus* and *Synechocystis* clones remain univestigated.

Darkness survival

Under conditions of total darkness but in inorganic culture medium replete with nutrients, both the marine species *Agmenellum quadruplicatum* and *Anacystis marina* showed high potential for survival without significant growth, both species being able to resume growth from replacement in light after spending at least 24 wk in darkness at 20°C (Antia and Cheng 1970). A further study of the effects of temperature on the darkness survival indicated that *A. quadruplicatum* survived longer at 20°C (35 wk) than at 2°C (23 wk), while *A. marina* survived longer at 2°C (52 wk) than at 20°C (28 wk), which difference was attributed to the normally prevalent temperature of the locations whence these species were isolated (Antia 1976). We believe that such pronounced capacity for darkness survival may reflect the seasonal rhythm of winter darkness or sinking into aphotic zones experienced by these organisms. This viewpoint is supported by a recent finding of photosynthetically competent phytoplankton from the aphotic zone (1000 m depth) of the Costa Rica Dome in the eastern tropical Pacific Ocean (Platt et al. 1983b).

Eucaryotes

Picoplankton-sized eucaryotes have been little investigated for their physiology and biochemistry. Apart from the possible consequences of their extremely small size (see Banse 1976), we do not expect much difference between them and their counterparts in the nanoplankton size range. Aside from the case of the eustigmatophytes hitherto known to contain chlorophyll *a* only, their possession of chlorophyll *b* or *c* (and the latter in combination with fucoxanthin or peridinin) as antennae pigments for capturing quanta (in place of the cyanobacterial phycobiliproteins) endows them with photosynthesis properties quite different from the cyanobacteria discussed in the previous section. However, these properties are believed to be a mere extension of those of their nanoplanktonic counterparts, which have been extensively and intensively studied for the past three decades. Several reviews of these studies have appeared in the literature of recent years, among which we recommend the reader to consult the monographs edited by Stewart (1974), Levandowsky and Hutner (1979–80), Falkowski (1980), Cox (1980), Morris (1980), and Platt (1981). Regarding the most up-to-date summaries of chloroplast pigments, the reader is referred to Jeffrey (1976), Wood (1979), and Andersen and Mulkey (1983) for the chlorophylls and to Liaaen-Jensen (1978) and Foss et al. (1984) for the carotenoids.

The chloroplast pigment composition (chlorophyll $a + b$ or $a + c$) of the eucaryotic picoplankters is considered responsible for their numerical predominance at the 0.5% light level (bottom of the euphotic zone), actually below the peak of *Synechococcus* populations (Glover et al. 1986). According to these investigators, such pigment composition confers greater photosynthetic and growth efficiencies on the eucaryotes in the dim blue-violet light occurring at the bottom of the euphotic zone, while the phycoerythrin-rich *Synechococcus* cells are favored by the green light available at the higher 1% light level in the euphotic zone. This conclusion was reached from observing ratios of fluorescence emission produced by excitation at 530 nm (green) to that produced by excitation at 470 nm (blue) for eucaryotes (0.3–0.7) relative to *Synechococcus* clones with Type I phycoerythrin (2.5–2.6) or clones with Type II phycoerythrin (5.2–5.4). Apart from spectral preference, evidence was also obtained for higher photosynthetic efficiencies and faster growth rates of the eucaryotes in low fluxes of the filtered blue-violet light in contrast with best performance of the *Synechococcus* clones in comparable fluxes of the filtered green light (Glover et al. 1986).

VI. Role in Primary Production of Freshwater and Marine Systems

Central to the question of how important are photoautotrophic picoplankton to total carbon production in lakes and oceans is consideration of methods used to estimate algal carbon assimilation. Earliest methods utilized either CO_2 or O_2 changes in small bottles (300–500 mL) (Gran 1931; Riley 1941), and production estimates so obtained would have included the complete plankton community, including algal picoplankton. With the advent of radiocarbon (^{14}C) tracer technology to address algal photosynthesis in aquatic systems (Steeman-Nielsen 1952) came the requirement for postfiltration following incubation, and with it the possibility of exclusion of picoplankton if too coarse a screen or filter was used. However, most early workers recognized the possibility of fine particle loss through some grades of glass-fiber filters (Harvey 1950), and therefore either collodion (0.5-μm pore size) or Millipore HA (0.45-μm pore size) membrane filters were initially recommended and most commonly used (Steeman-Nielsen 1952; Strickland 1960). Thus, it appears likely that most of the published ^{14}C-based estimates of carbon production in lakes and oceans where membrane filters were used have included the algal picoplankton contribution. However, Li et al. (1983) have noted that use of filters not able to trap particles at least 0.2 μm or smaller may underestimate production, and their observations have also been confirmed by Cynar et al. (1985), who noted passage of nanoplankton through 0.6- and 0.4-μm filters. In this regard we would recommend that a membrane filter of 0.2-μm nominal pore diameter become the standard filter for use with the ^{14}C technique.

The now generally accepted and broadly applied use of the ^{14}C method to estimate phytoplankton production in aquatic systems has, however, introduced a host of additional problems and associated sources of error that have been the subject of much debate and recent review (Sorokin 1971; Banse 1974; Verduin 1975; Eppley and Peterson 1979; Gieskes et al. 1979; Parsons et al. 1980; Stockner et al. 1980; Peterson 1980; Harris 1980; Carpenter and Lively 1980). Recent studies of CO_2 and O_2 flux (Johnson et al. 1981, 1983), O_2 production (Shulenberger and Reid 1981), O_2 consumption (Jenkins 1982; Jenkins and Goldman 1985), and nutrient injection (Holligan et al. 1985) have provided new evidence for higher oceanic carbon production rates, some estimates of which are up to an order of magnitude higher than values derived by the ^{14}C method (Kerr 1983). The concurrent use of multiple methods to assess carbon production in the oligotrophic ocean is now underway, including consideration of the now generally accepted contribution made by algal picoplankton to total oceanic production (Li et al. 1983; Williams et al. 1983; Laws et al. 1984).

Most of the early attempts to discern the relative contributions by net- and nanophytoplankton size fractions to total carbon production in lakes (Rodhe et al. 1958; Gliwicz 1967; Kalff 1972; Gelin 1975) and oceans (Wood and Davis 1956; Holmes and Anderson 1963; Anderson 1965; Malone 1971; McCarthy et al. 1974; Durbin et al. 1975; Beers et al. 1975) found the majority of biomass and carbon production to be in the nanoplankton fraction, which investigators most often considered to be composed of cells ranging from <20 to 50 μm in diameter. Although some have questioned the particle separation and filtration efficiency of membrane filters (Sheldon 1972), with the introduction in the mid-1970's of polycarbonate filters with their improved resolution of nominal pore diameter, it was possible to size-fractionate plankton more accurately and for the first time study the relative contributions of heterotrophic and autotrophic picoplankton to total carbon production and biomass in aquatic ecosystems.

In marine systems, Saijo (1964) and Saijo and Takesue (1965), working in the Indian Ocean and South China Sea, were the first to show measurable carbon production by algal picoplankton passing an 0.8-μm membrane filter, but little was made of their discovery and nearly a decade would pass before Berman (1975), in a study in the Gulf of California, would again draw attention to the possible importance of very small phytoplankton (0.2–3.0 μm fraction) to total autotrophic biomass and production and emphasize their potential contribution to total oceanic carbon production. Studies by Throndsen (1976) and Throndsen and Heimdal (1976) in coastal marine waters of Norway would again demonstrate the significant contribution of small (<5 μm) "ultraplankton" to total carbon production, and their findings, resulting in a summary paper by Throndsen (1979), would strongly urge marine scientists to begin detailed studies of this very important "phytoplankton fraction." Gieskes et al. (1979), although primarily concerned with identifying possible sources of error related to use of the ^{14}C technique to estimate primary production, found significant production by algal picoplankton size fractions (<1 and <3 μm) in the Atlantic North Equatorial Current, but they drew no conclusions from these findings about their possible importance to total carbon production in the oligotrophic oceans. Bienfang and Szyper (1981), working in the subtropical Pacific, recognized the large contribution (±80%) made by "small particles" to both biomass and production, but their fractionation procedure included all particles <5 μm, and the contribution by picoplankton per se could not be separated from that by small nanoplankton (3–5 μm). Herbland and Le Bouteiller (1981), although concluding that very small photosynthetic particles exist in the Equatorial Atlantic, did not elaborate on their potential significance, and they considered the "small particles" (<3 μm) to be inactive photosynthetic organisms. Larsson and Hagström (1982), working in a eutrophic gradient in the Baltic Sea to ascertain the significance of phytoplankton exudation as a carbon source for bacterial production, found picoplankton to be a very significant contributor to both biomass and production, particularly during periods of nutrient depletion. By 1983 the contribution of photoautotrophic picoplankton to the total carbon budget of the world's oceans had been firmly established by studies on the subtropical and tropical Pacific (Li et al. 1983; Bienfang and Takahashi 1983; Takahashi and Bienfang 1983; Laws et al. 1984), Central Pacific gyre (Iturriaga and Mitchell 1986), Atlantic shelf (Douglas 1984), tropical Atlantic (Platt et al. 1983a; Glover et al. 1985b, 1986), Celtic Sea (Joint and Pomroy 1983; Joint et al. 1986), Arctic Ocean (Smith et al. 1985), Mediterranean Sea (Berman et al. 1984), and in coastal waters of Japan (Takahashi et al. 1985) and California (Putt and Prezelin 1985).

It was Rodhe (1955) who first drew the attention of limnologists to the possible importance of small "μ-algae" to the carbon metabolism of north-temperate oligotrophic lakes. He found small (1–2 μm) green algae in great abundance during winter in subarctic lakes of Swedish Lapland and speculated on their significance as a food source for overwintering zooplankton populations, but warned of the difficulty to enumerate them by use of the conventional Utermöhl settling technique.

Nearly 10 yr would pass before investigators working in eutrophic Loch Leven, Scotland, would report on the occurrence and high gross photosynthesis of a bloom forming small *Synechococcus*-like, rod-shaped cyanobacteria that to the untrained eye could easily have been mistaken for bacteria (Bailey-Watts et al. 1968). These important first discoveries of algal picoplankton in freshwater failed to stimulate further research on lake picoplankton and, instead, interest turned to nanoplankton, more easily dealt with by the conventional screens and filters in use at the time. Early work on size-fractionated production in lakes documented the importrant contribution made by small (<20 μm) nanophytoplankton to total carbon production in lakes of varying trophic state (Rodhe et al. 1958; Gliwicz 1967; Gelin 1971, 1975; Gelin and Ripl 1978).

Finer separation procedures were first used by Kalff (1972) working at Lac Hertel, Quebec, who found up to 15% of total ^{14}C activity had passed a 5-μm filter but was retained by a 0.45-μm filter. However, he attributed this observation to cell breakage or "leakage" and did not comment on the possibility of production by algal picoplankton or small nanoplankton cells. It was Paerl (1977) and Paerl and MacKenzie (1977) who first used separation and retention by 3.0- and 0.2-μm Nuclepore filters and autoradiography procedures to demonstrate the significant contribution made by autotrophic picoplankton (Paerl's LRGTs = little round green things) to both biomass and carbon assimilation in a variety of New Zealand lakes, but only relative (percentage) ^{14}C activities were given, with the highest percentage contribution by picoplankton most often found in the oligotrophic lakes. Working on photosynthesis and extracellular release rates from fractionated plankton samples from Lakes Erie and Ontario, Lee and Nalewajko (1978) found high uptake and organic release by the 0.45−3 μm size fraction, especially so in the less productive Lake Ontario. They considered these results to be related more to the passage of large cells through the 3-μm Nuclepore filters than to production and release by small autotrophic picoplankton. Costella et al. (1979), working in Great Central Lake, British Columbia, were the first to publish in situ rates of primary production by picoplankton passing a 3-μm Sartorius membrane filter, commenting on the possible importance of these small "ultraplankton" to whole-lake metabolism. Munawar and Fahnenstiel (1982) and Fahnenstiel et al. (1986) found that phototrophic picoplankton are an important component of the phytoplankton community of Lake Superior, contributing up to 50% of both total carbon production and total chlorophyll biomass. Stockner (1987), working on several coastal warm monomictic lakes in British Columbia, confirmed the initial study by Costella et al. (1979) and, using 0.2−3.0 μm Nuclepore fractionation procedures, demonstrated the large contribution made by algal picoplankton to both daily and annual total carbon production, while speculating on the possible role of picoplankton in food webs of these ultraoligotrophic lakes.

Table 6 summarizes published estimates of photoautotrophic picoplankton growth, production,and biomass from both freshwater and marine ecosystems. Hourly carbon production rates for marine picoplankton ranged from a low of 0.01 mg $C \cdot m^{-3} \cdot h^{-1}$ reported by Saijo and Takesue (1965) to over 31 mg $C \cdot m^{-3} \cdot h^{-1}$ observed by Glover et al. (1985a). The percentage contribution by phototrophic picoplankton to total carbon production ranged from 1 to 90%, with a tendency for substantially higher contributions in more oligotrophic regions of the world's oceans. Estimates of algal picoplankton biomass (usually as chlorophyll) were less variable than production estimates, ranging from 0.05 to 1.0 mg $Chl \cdot m^{-3}$ and contributing to 1 to 90% to total chlorophyll biomass. Published assimilation numbers (milligrams carbon per milligram chlorophyll per hour) for algal picoplankton were generally high with values ranging from 0.3 to 14.5, indicative of a rapid turnover of carbon per unit chlorophyll. There were fewer measurements of specific growth rates (μ) reported (see Putt and Prezelin 1985) but values, ranging from 0.15 to $8.9 \cdot d^{-1}$, tended to be considerably higher in upwelling and neritic regions than in tropical and subtropical ocean (Li et al. 1983; Landry et al. 1984).

Rates of picoplankton carbon production from freshwater are very similar to marine values, ranging from 0.1 to 7.0 mg $C \cdot m^{-3} \cdot h^{-1}$, representing from 16 to 70% of total carbon production (Table 6). Biomass of phototrophic picoplankton contributed from 6 to 43% to total chlorophyll, with values ranging from 0.29 to 1.0 mg $Chl \cdot m^{-3}$. Specific growth rates have been determined on cyanobacterial picoplankton in Lake Superior and for September 1983 were $1.5 \cdot d^{-1}$ in the epilimnion and $0.8 \cdot d^{-1}$ in the hypolimnion (Fahnenstiel et al. 1986). Assimilation numbers reported by Costella et al. (1979) and Munawar and Fahnenstiel (1982) were similar, ranging from 2.0 to 2.7 mg $C \cdot mg\ Chl^{-1} \cdot h^{-1}$, well within the range of marine estimates.

In both freshwater and marine ecosystems, the algal picoplankton contribution to total primary production tended to increase with depth in the euphotic zone (Li et al. 1983; Platt et al. 1983a; Glover et al. 1985a, 1985b; Costella et al. 1979; Craig 1984), reflecting, in part, the greater efficiency of the photopigments of both procaryotic and eucaryotic picoplankton to utilize blue-green light (Li et al. 1983; Wood 1985; Glover et al. 1985a). Glover et al. (1985b) noted higher algal picoplankton carbon assimilation rates in surface waters just after dawn and before dusk with apparent photoinhibition during midday on bright days, indicating greater photosynthetic efficiency at periods of low photon flux. In contrast, Putt and Prezelin (1985) observed peak production in midday for cyanobacterial picoplankton in the Santa Barbara channel, noting, however, that this result was not surprising considering that photosynthesis was largely light limited for the irradiance at 30 m.

The measured rates of algal picoplankton carbon production seem to show little variation along eutrophic gradients (Larsson and Hagström 1982) or among coastal, slope, and open ocean stations (Glover et al. 1985b; Glover 1985), but the percentage contribution by phototrophic picoplankton to total carbon production is considerably higher in the oligotrophic open ocean (50−80%) than in the mesoeutrophic coastal marine waters (2−25%). A similar trend was also noted in lakes of varying trophic states (Stockner 1987; Fahnenstiel et al. 1986; Craig 1984), with the exception of some episodic midsummer cyanobacterial picoplankton blooms that seem to occur in some eutrophic lakes (Bailey-Watts et al. 1968; Cronberg and Weibull 1981). Very recently, Joint et al. (1986) reported the only described seasonal pattern of algal picoplankton fluxes in temperate marine waters, noting that the maximum production rates and relative contribution to total phytoplankton production occurred in midsummer (August).

VII. Role in Aquatic Food Webs

Only 15 yr ago the prevailing opinion among aquatic micro-

TABLE 6. Literature summary of size-fractioned production and biomass variables of algal picoplankton in

Location	Fraction and filter	Growth (d^{-1})	Assimilation (mg C·mg Chl^{-1}·h^{-1})	Production rate	Total production (%)	Biomass
LAKES						
1. Great Central, British Columbia, Canada	0.45–3.0 μm Sartorius	—	2.73	0.10–0.73 mg C·m^{-3}·h^{-1} 24–34 mg C·m^{-3}·d^{-1}	16	0.5–1.0 mg Chl·m
2. Superior	0.45–3.0 μm Selectron and Nuclepore	—	2.0	0.58 mg C·m^{-3}·h^{-1}	47	0.29 mg Chl·m
3. Hertel, Quebec Canada	0.45–5.0 μm Millipore SM	—	—	7.0–18.0 mg C·m^{-3}·d^{-1}	5–60	3–205 mg·m^{-}
4. Coastal Lakes, British Columbia, Canada	0.2–3.0 μm Nuclepore	—	—	0.3–7.0 mg C·m^{-3}·h^{-1}	20–70	—
5. Superior	0.22–1.0 μm Millipore and Nuclepore	1.5	—	0.31 mg C·m^{-3}·h^{-1}	16	—
6. South Carolina reservoirs	0.4–3.0 μm Nuclepore	—	—	—	16–40	—
OCEANS						
1. Baltic Sea	0.2–3.0 μm Nuclepore	—	0.03–0.22	0.2–3.4 mg C·m^{-3}·h^{-1} 36–44 g C·m^{-2}·yr^{-1}	3–43	6.4–26 mg C·m
2. Atlantic, Nova Scotia shelf	0.2–1.0 μm Nuclepore	0.9–8.9[b]	—	1.8–5.8 mg C·m^{-3}·h^{-1}	12–65	0.45–5. mg C·m
3. Pacific, tropical	0.2–1.0 μm Nuclepore	—	—	0.2–4.5 mg C·m^{-3}·h^{-1}	20–80	0.05–1. mg Chl·m
4. Atlantic, Arctic	0.2–1.0 μm Nuclepore	—	0.1–0.8	0.12–2.0 mg C·m^{-3}·h^{-1}	10–25	0.26–0.5 mg Chl·m
5. Indian, S. China Sea	0.45–0.8 μm Millipore HA	—	0.9–2.3	0.01–0.08 mg C·m^{-3}·h^{-1}	1–22	0.07–.40 mg Chl·
	0.8–5.0 μm Millipore AA	—	0.7–2.5	0.02–0.16 mg C·m^{-3}·h^{-1}	9–40	0.05–.30 mg Chl·m
6. Celtic Sea	0.2–2.0 μm Nuclepore	—	—	0.7–12.4 mg C·m^{-3}·h^{-1}	20–30	—
7. Pacific, subtropical	0.2–3.0 μm Nuclepore	1.3–2.5	5–14.5	0.75–1.5[c] mg C·m^{-3}·h^{-1}	—	0.8–0.1 mg Chl·m
8. Pacific, Coastal Japan	0.2–3.0 μm Nuclepore	—	—	—	—	0.25–0.5 mg Chl·m
9. Pacific, subtropical, Hawaii	0.45–5.0 μm Nuclepore	—	—	7.6 mg C·m^{-3}·h^{-1}	83	0.1–0. mg Chl·m

nkton in freshwater and marine ecosystems.

Biomass	Total biomass (%)	Trophic state	Comments	Reference(s)
0.5–1.0 mg Chl·m^{-3}	6	Oligotrophic	Seasonal and depth integrated averages	Costella et al. 1979
0.29 mg Chl·m^{-3}	43	Oligotrophic	Seasonal and depth integrated averages	Munawar and Fahnenstiel 1982
3–205[a] mg·m^{-3}	2–32	Eutrophic	Seasonal average	Kalff 1972
—	—	Oligotrophic	Seasonal average	Stockner 1987
—	—	Oligotrophic	Epilimnion average	Fahnenstiel et al. 1986
—	0.2–37	?	Seasonal and site average for 2 m depth	Tison and Wilde 1981
6.4–26.8 mg C·m^{-3}	10–25	Eutrophic gradient	Seasonal average	Larsson and Hagström 1982
0.45–5.9 mg C·m^{-3}	—	Trophic gradient	June 5–11, 1982 (5 stations)	Douglas 1984
0.05–1.0 mg Chl·m^{-3}	25–90	Oligotrophic	Mar.–Apr. 1981	Li et al. 1983
0.26–0.54 mg Chl·m^{-3}	10–70	Oligotrophic	Aug.–Sept. 1981	Smith et al. 1985
0.07–.40 mg Chl·m^{-3}	1–39	Oligotrophic	Oct.–Jan. 1963–64	Saijo and Takesue 1965
0.05–.36 mg Chl·m^{-3}	5–40	Oligotrophic		
—	—	Coastal trophic gradient	Aug.–Oct. 1982	Joint and Pomroy, 1983
0.8–0.14 mg Chl·m^{-3}	70	Oligotrophic	Nov.–Dec. 1980	Bienfang and Takahashi 1983; Takahashi and Bienfang 1983
0.25–0.50 mg Chl·m^{-3}	20–100	Trophic gradient	July–Aug., Dec. 1983	Takahashi et al. 1985
0.1–0.2 mg Chl·m^{-3}	80	Oligotrophic	Oct. 1978 – Dec. 1979	Bienfang and Szyper 1981

TABLE 6. (*Concluded*)

Location	Fraction and filter	Growth (d^{-1})	Assimilation (mg C·mg Chl^{-1}·h^{-1})	Production rate	Total production (%)
10. Atlantic, tropical	0.4–1.0 μm Nuclepore	0.15	0.68	0.50[d] mg C·m^{-3}·h^{-1}	50–60
11. Atlantic, N. equatorial current	0.4–1.0 μm Unipore	—	—	0.66–1.30 mg C·m^{-3}·d^{-1}	22–30
	1.0–3.0 μm Unipore	—	—	0.48–0.69 mg C·m^{-3}·d^{-1}	16
12. Atlantic, Gulf of Maine – Sargasso Sea	0.2–3.0 μm Nuclepore	—	—	3.1–31 mg C·m^{-3}·h^{-1}	60–80
13. Atlantic, equatorial	0.45–3.0 μm Nuclepore	—	2.1	1.08 mg C·m^{-3}·h^{-1}	20
14. Pacific, coastal Hawaii	0.22–0.3 μm Nuclepore	1.4–2.0[e]	—	—	—
15. Mediterranean Sea	0.2–3.0 μm Nuclepore	—	—	2.5–9.2[f] mg C·m^{-3}·h^{-1}	25–90
16. Oslofjorden, Norway	0.45–5.0 μm Nytal gauze and Sartorius	—	—	0.1–30.0 mg C·m^{-3}·h^{-1}	15–90
17. Atlantic northwest	0.2–1.0 μm Nuclepore	—	—	0.01–15.7 mg C·m^{-3}·d^{-1}	0.1–66
				56.1–338.8 mg C·m^{-3}·d^{-1}	15–62
18. Pacific Ocean, coastal California	0.2–5.0 μm[h] Nuclepore	0.7–1.1	1.8–2.1	1.29–2.19 mg C·m^{-3}·h^{-1}	75
19. Pacific Ocean, North Central Gyre	0.45–2.0 μm Millipore HA, Whatman 934 AH	1.8	—	1.86 mg C·m^{-3}·h^{-1}	57

[a]Biomass from Utermohl counts.
[b]Specific growth rate = [carbon uptake (μg C·L^{-1}·d^{-1})]/[cell carbon (μg C·L^{-1})].
[c]Value from nutrient enrichment experiment.
[d]Maximum values.
[e]Data of Landry et al. (1984).
[f]Maximum values.
[g]Estimate from Fig. 6.
[h]Although small nanoplankton are included, authors comment on dominance by cyanobacteria.

Total duction (%)	Biomass	Total biomass (%)	Trophic state	Comments	Reference(s)
–60	1.09[d] mg Chl · m^{-3}	44	Oligotrophic	July 1982	Platt et al. 1983a
–30	—	—	Oligotrophic	Sep.–Oct. 1977	
16	—	—	Oligotrophic	Nov.–Dec. 1978	Gieskes et al. 1979
–80	7–16 mg Chl · m^{-2}	60–97	Oligotrophic	Aug. 1983	Glover et al. 1985b
20	0.48 mg C · m^{-3}	43	Oligotrophic	Feb. and Apr. 1979	Herbland and Le Bouteiller 1981
—	0.22 mg Chl · m^{-3}	60	Oligotrophic	Aug.–Sept. 1982 (avg. 6 stn.)	Laws et al. 1984
–90	10.8–14.4[f] mg Chl · m^{-2}	60–80	Oligotrophic	1981–83 (avg. 5 cruises)	Berman et al. 1984
–90	—	—	Trophic gradient	Seasonal average	Throndsen 1978
–66	0.05–1.4 mg Chl · m^{-3}	10–50	Trophic gradient	July–Aug. 1984	Glover et al. 1986
–62	3.7–37.3 mg Chl · m^{-2}	16–62			
75	0.09–1.35 mg Chl · m^{-3}	11–93	Coastal trophic gradient	Sep. 1983	Putt and Prezelin 1985
57	—	—	Oligotrophic	Average 6 depths (G–105 m)	Iturriaga and Mitchell 1986

acteria.

biologists was that nearly all bacteria in lakes and oceans were associated with particles and that their role as "decomposers" was to remineralize particulate and dissolved organic matter and recycle essential plant nutrients (Wood 1965). Hobbie et al. (1972) and later Azam and Hodson (1977) were among the first to provide unequivocal evidence for the overwhelming abundance and ubiquity of free-living bacteria in aquatic systems. The current literature now supports the perception of free-living bacteria serving as a principal food source for a wide variety of metabolically active, heterotrophic microorganisms (protozooplankton) that act through the process of feeding as the principle remineralizers of organic matter and recyclers of nutrients in aquatic systems (Johannes 1965; Pomeroy and Johannes 1966; Pomery 1974, 1984; Wiiliams 1981, 1984; Joint and Morris 1982; Azam et al. 1983; Azam and Ammerman 1984; Sherr and Sherr 1983; Ducklow 1983; Porter et al. 1985). In addition, bacterivory has been reported for several oceanic algal nanoflagellates, so that protists with and without chloroplasts can occupy overlapping trophic roles (Estep et al. 1986). A prevailing question germane to this review that is now being asked is: how do phototrophic picoplankton fit within the framework of this new paradigm?

We now know that algal picoplankton are much less abundant than free-living bacteria in the euphotic zone of lakes and oceans; probably from 5 to 20% of the total bacterial numbers would be a realistic estimate. However, because they are, on average, considerably larger than free-living bacteria, they can make a higher contribution (from 20 to 50%) to the total picoplankton biomass and may contribute up to 20% to the total microbial plankton biomass in the open ocean (pico-, nano-, microplankton) (Johnson and Sieburth 1979). As has already been discussed in previous sections of this review, it is now quite clear that algal picoplankters are a metabolically active part of the microbial plankton community, particularly in more oligotrophic conditions where they contribute significantly to the total daily and annual carbon production in lakes and oceans. Like bacteria, the relative constancy of their numbers during the growing season implies control by predation (Johnson et al. 1982; Campbell 1985; Iturriaga and Mitchell 1986). What we do not know, however, is the degree to which they contribute to pelagic food webs, either as direct links or important sinks in the movement of carbon to higher trophic levels.

Flagellates

Based largely on physical considerations, small heterotrophic microflagellates, falling within the nanoplankton size range (2−20 μm), should be optimally suited for grazing on free-living bacteria and algal picoplankton in the euphotic zone of lakes and oceans, particularly so in more eutrophic situations where their densities tend to be higher (Fenchel 1982a, 1982b, 1984; Azam et al. 1983; Davis and Sieburth 1984; Gast 1985). In Villefranche-sur-Mer, Laval (1971) was one of the first to note that minute picoalgae were ingested by choanoflagellates, and nearly a decade later, Perkins et al. (1981) observed direct utilization of a marine *Synechococcus* picoplankter by heterotrophic flagellates in Chesapeake Bay. Johnson et al. (1982), using TEM to analyze field plankton collections, found cyanobacterial picoplankton in food vacuoles of phagotrophic nanoplankton, mostly in dinoflagellates and choanoflagellates from Georges Bank, collected at a time when cyanobacterial populations were the highest observed by them (10^5 cells·mL^{-1}). In the laboratory, the flagellate *Actinomonas* was maintained in culture for over a year using an oceanic isolate of *Synechococcus* as food, and TEM analysis confirmed the direct utilization and assimilation by the flagellate (Johnson et al. 1982). In a laboratory chemostat study, Parslow et al. (1986) recorded growth rates of $2 \cdot d^{-1}$ for the heterotrophic flagellate *Pseudoboda* feeding on the eucaryotic picoplankter *Micromonas pusilla*.

In the Laurentian Great Lakes, Caron et al. (1985b), Fahnenstiel et al. (1986), and Boraas et al. (1985), working on Lakes Ontario, Superior, and Michigan, respectively, reported on the frequent occurrence of chrococcoid cyanobacteria in the food vacuoles of heterotrophic microflagellates, and each commented on the potential importance of algal picoplankton as a food source for flagellates and other larger microzooplankton. Recent studies from freshwater systems have also shown that the common phototrophic flagellates (e.g. *Cryptomonas*, *Ochromonas*, *Gymnodinium*) can also graze on picoplankton (Porter et al. 1985; Bird and Kalff 1986; Sanders et al. 1985). Bird and Kalff (1986) also noted that several species of the genus *Dinobryon* have the potential to remove more bacteria from the water column than crustaceans, rotifers, and ciliate consumers combined and at grazing rates equivalent to those observed for marine heterotrophic microflagellates. Although these studies imply bacterial grazing by these phototrophic algae, there is little reason to doubt that small algal picoplankton can also be utilized, and it is likely that they, too, are an important component of the diet of these mixotrophic flagellates.

One can now safely conclude that the direct utilization of both heterotrophic and autotrophic picoplankton by flagellates in both marine and freshwater ecosystems has been well documented and that, as predators, flagellates provide a direct and tightly coupled link for the transfer of carbon from the pico- to nanoplankton-sized particles which tend to be more readily grazed by larger microzooplankton and metazoans in the pelagial. However, recent reports by Suttle et al. (1986) from freshwater and Goldman and Caron (1985a) from marine waters demonstrate that some microflagellates are not obligate "picovores," since they possess the ability to feed on much larger nano- and microplankton algae as well, thereby acting as potential sinks by removing carbon that is normally more readily available to larger metazoan grazers.

From the above, it now appears that microflagellates are likely the most important grazers of algal picoplankton in aquatic ecosystems and that, as predators, with a high assimilation efficiency and rapid growth potential, they serve to keep heterotrophic and autotrophic picoplankton in an active growth phase by their cropping and subsequent release of dissolved organic matter and inorganic nutrients (Goldman et al. 1985; Goldman and Caron 1985b).

Ciliates and Other Microzooplankton

The abundance of microzooplankton (20−200 μm) and their active metabolic role in the pelagial plankton has long been recognized (Johannes 1965; Parsons and Le Brasseur 1970; Sorokin 1971; Sorokin and Paveljeva 1978), but the important role they play in the emerging new paradigm has only recently been emphasized (Pomeroy 1974; Sieburth and Davis 1982; Burkill 1982; Landry and Hassett 1982; Taylor 1982; Gifford 1985).

Apart from a laboratory culture study, where Johnson et al.

(1982) maintained the ciliate *Uronema* on a strain of marine *Synechococcus*, there are few reported direct or even circumstantial accounts of algal picoplankton utilization by marine or freshwater ciliates. Fenchel (1984) concluded that the majority of ciliates in the open ocean are, in fact, not bacterivorous, feeding largely on heterotrophic and phototrophic nanoplankton. However, in more eutrophic coastal regions such as estuaries and fjords, bacterivory is likely of greater importance for ciliates and other microzooplankton (Gast 1985; Porter et al. 1985). The larger size of algal picoplankton makes them more suitable for ingestion by ciliates than the smaller (0.4–0.6 μm) free-living bacteria; however, their lesser abundance coupled with their small size may also impart some degree of refuge from ciliate grazing (Banse 1982). Nevertheless, small aloricate ciliates were recently reported as significant potential consumers of both bacteria and chroococcoid cyanobacteria (Sherr et al. 1986), and in a recent study by Iturriaga and Mitchell (1986), using ^{14}C-labelled chroococcoid cyanobacteria and microautoradiography, they observed direct utilization and assimilation by several protozoan grazers, many of which were ciliates. Grazing was observed to be highest near the deep chlorophyll maximum where production and consumption (30–40% of standing crop per day) were highest. Further research on ciliate grazing is required to quantify the degree of algal picoplankton utilization, but the picture emerging from both freshwater (Porter et al. 1979; Pace and Orcutt 1981; Pace 1982; Gates 1984) and marine systems (Rassoulzadegan and Etienne 1981; Burkill 1982; Rassoulzadegan 1982; Capriulo and Ninivaggi 1982; Stoecker and Evans 1985; Taylor et al. 1985; Porter et al. 1985) is one with ciliates contributing from 16 to 32% to the total biomass of marine microzooplankton communities (Taylor 1982) and, with their potential for rapid growth (Banse 1982), ciliates playing an important role in the microbial food webs as predators of microflagellates and as grazers of both heterotrophic and autotrophic picoplankton.

The central role of microzooplankton in food webs of lakes and oceans appears to be as grazers of both heterotrophic and phototrophic nanoplankton with some utilization of picoplankton, but with far less efficiency than by flagellates or ciliates. Algal picoplankton is within the size range likely to be utilized by nauplii and early copepedite stages of copepods in lakes and oceans and by planktonic rotifers in lakes. Caron et al. (1985b) observed ingested chroococcoid cyanobacteria in the rotifer *Keratella cochlieris* Lake Ontario, inferring that they were digested and hence being utilized as food. Similar use of chroococcoid cyanobacteria as food by a variety of rotifer species has been observed by E. A. MacIsaac (unpubl. data) in British Columbia coastal lakes. Rotifers would also be expected to be important predators of flagellates, small ciliates, and other nanoplankton.

As was the case with flagellate predation on picoplankton, grazing by ciliates and other microzooplankton also contributes to the dissolved organic matter pool for utilization by bacteria, and also to the release and recycling of inorganic nutrients for bacteria and phytoplankton (Johannes 1965; Taylor 1982; Caron et al. 1985a).

Metazoans

The literature on freshwater cladoceran grazing as related to particle size would tend to support utilization of algal picoplankton, albeit less efficiently than larger nanoplankton prey (Peterson et al. 1978; Porter 1984). Chroococcoid cyanobacteria have been seen in the gut of *Eubosmina* in coastal British Columbia lakes, but they appeared to be undigested (E. A. MacIsaac, unpubl. data). The passage of intact, viable bacteria through the guts of zooplankton is common (Porter 1984), but further research is required to verify whether algal picoplankton are digested by cladocerans. If they are, they would serve as an important direct link to higher trophic levels because cladocerans are most often the preferred prey item of planktivorous fish in lakes (Lynch 1979; Stockner 1987).

Apparently some copepods cannot digest algal picoplankton because undigested and presumably viable cells have been found in copepod fecal pellets in both marine (Silver and Alldredge 1981; Johnson et al. 1982; Iturriaga and Mitchell 1986) and freshwater systems (Caron et al. 1985b). Since *Synechococcus* has also been found associated with a variety of oceanic macroaggregates that have sunk from the euphotic zone (Glover 1985), it is likely that this particle-bound transport (feces and aggregates) is the principle means of transfer of algal picoplankton carbon from the pelagic to the abyssal food web in oceans. A similar export of algal picoplankton associated with phytodetritus and fecal pellets is also important in lakes, serving as a food source for the zoobenthic community. We are not aware of reports documenting the presence of algal picoplankton in sediment traps or in the guts of benthic invertebrates in lakes that would verify the importance of this transport from the euphotic zone, but the losses are not great; according to Bishop et al. (1980), only 1–10% of primary production is lost to the bathy–pelagial from the open ocean, but higher losses are reported for lakes (Shapiro et al. 1971; Edmondson and Lehman 1981).

There are a host of other metazoan filter feeders in the marine environment that have the potential to retain particles from 1 to 2 μm in diameter, including ascidians, bryozoans, pelagic larval stages of marine invertebrates, bivalves, and sponges. Heaviest grazing by these metazoans would likely occur in estuaries and in nearshore coastal waters because it is at these locations where the greatest abundance and biomass of picoplankton occur (Johnson and Sieburth 1982; Gast 1985; Glover 1985). The appendicularian *Oikopleura* can effectively filter-feed on bacteria (King et al. 1980) and would likely utilize algal picoplankton, as well, although these authors do not report such use. Iturriaga and Mitchell (1986) observed considerable numbers of chroococcoid cyanobacteria in the cephallic region of a chaetognath, suggesting direct utilization by what is normally considered a primary carnivore. Further research is necessary to quantify the significance of algal picoplankton use by a variety of metazoans in lakes and oceans, but the evidence in hand strongly suggests population control by predation.

Food Webs

Just as particle physicists in search of the ultimate form of matter discovered complexity instead of simplicity, and a strong interconnectedness among both living and nonliving matter (Zukav 1979), so also, within the present decade, aquatic microbiologists and ecologists, armed with new means of observation (TEM, SEM, epifluorescence microscopy) and measurement (Nuclepore filters, ATP), have discovered incredible complexity and interrelatedness in the realm of the minute life forms (Sieburth 1982; Azam et al. 1983; Pomeroy 1984). Within the last 5 yr there have been many comprehensive reviews of aquatic bacteria and protozoans in the marine environment, and each has attempted to reconcile the role of

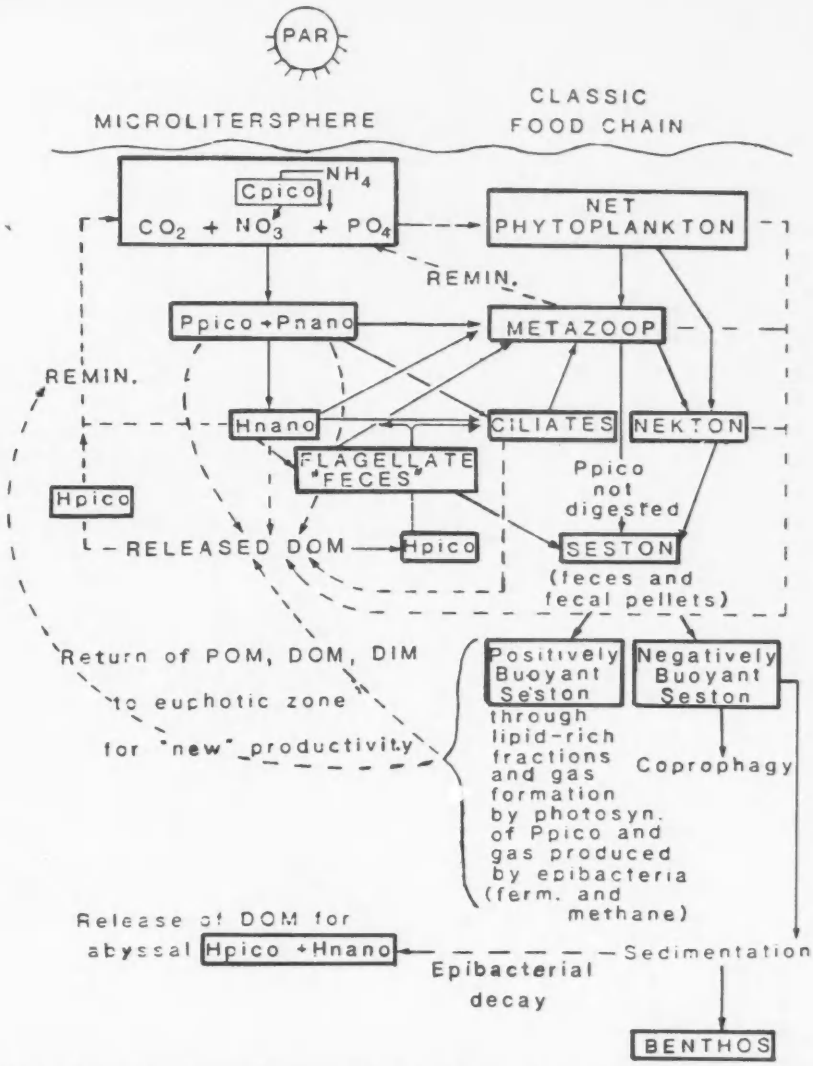

FIG. 6. Model of how numerous components of picoplankton and nanoplankton form three trophic-mode microcosms in just a few microlitres of water to create the basic foundation of life in oligotrophic waters and how this understudied part of the food web interfaces with the classic diatom–copepod–fish food chain. PAR = photosynthetically active radiation; Cpico = chemolithotrophic picoplankton; Ppico = phototrophic picoplankton; Pnano = phototrophic nanoplankton; Hpico = heterotrophic picoplankton; Hnano = heterotrophic nanoplankton; POM = particulate organic matter; DOM = dissolved organic matter; DIM = dissolved inorganic matter. (Reproduced with permission from Sieburth and Davis 1982)

each of these groups of microorganisms within the framework of the emerging new paradigm (Azam et al. 1983; Pomeroy 1984). In Fig. 6 we present the model of the marine food web initially proposed by Sieburth and Davis (1982). Although intended to conceptually model processes in the pelagic of the oligotrophic ocean, we consider it equally appropriate for application to large oligotrophic or mesotrophic lakes, such as the Laurentian Great Lakes in North America, whose physical and chemical processes show strong similarities with those of the world's oceans. In fact, we feel that after some accomodation is made for the prevalence of stronger links between microphytoplankton and larger metazoans in more eutrophic situations, the processes depicted in Fig. 6 are germane to all aquatic systems, irrespective of their prevailing trophy. However, further research is required to determine just how important the microbial food web is in waters of varying productivity, at different latitudes, and at different seasons because there are already reports that seem to cast some doubt on the importance of energy flows through the "microbial loop" (Joint and Williams 1985; Ducklow et al. 1986). We also need to better understand the implications of the "microbial loop" (Azam et al. 1983), the "spinning wheel" (Goldman 1984), and

the many "trophic triangles" (Stoecker and Evans 1985) on the productivity of pelagic and demersal fisheries resources of the great lakes and major oceans of the world, upon which mankind is so dependent. Studies in regions where the loop is inactive or "disengaged" will provide necessary answers to questions addressing whether the "loop" shunts carbon and dissipates energy through multiple transfers, thereby affecting production at higher trophic levels (Pomeroy and Deibel 1985). Certainly our most productive freshwater fisheries are based on species which can, in effect, "bypass" the microbial loop by feeding directly on aquatic macrophytes and phytoplankton (Melack 1976).

Miscellaneous Ecological Considerations

Many investigators have reported significant ^{14}C assimilation by picoplankton passing a 1-μm Nuclepore filter (Li et al. 1983; Platt et al. 1983a, 1983b; Douglas 1984; Smith et al. 1985; Glover et al. 1986), and Fuhrman and McManus (1984) have concluded that small marine eucaryotes are capable of passing a 0.6-μm filter, while Cynar et al. (1985) have found predaceous nanoplankton in both 0.6- and 0.4-μm filtrates. These findings will likely influence some of the interpretations of previous workers who used 3-μm Nuclepore filters to separate heterotrophic bacteria from phototrophic pico-, nano-, and microplankton (Derenbach and Williams 1974; Currie and Kalff 1984a, 1984b; Turley and Lochte 1985). We suggest that future studies, requiring separation of the heterotrophic from phototrophic component of the plankton, use as fine a membrane filter as is practical and use microscopy or culture techniques to assure the separation. In view of the above, we also wonder about the possibility of exclusion or inclusion of picoplankton when dialysis bags and cage cultures are used in studies of the biochemistry and ecology of phytoplankton (Sakshaug and Jensen 1978).

Further studies on rivers and smaller streams are necessary to augment studies by Meyer et al. (1985) on the role of bacteria and protozooplankton in the trophodynamics of these important lotic ecosystems. We wonder about the occurrence and importance of algal picoplankton in other habitats. Small coccoid cyanobacteria have been seen in microbial mats from thermal springs (Brock 1978), from shallow intertidal marine sediments (Stal and Krumbein 1985), and from saline lakes like Solar Lake, Sinai, where they are found in great abundance at certain seasons (Jorgensen and Revsbech 1983). Does the "microbial loop" operate at the temperature and light extremes of these harsh environments? Are algal picoplankton and other microheterotrophs tightly coupled to bacterial production and nutrient recycling in small streams, hot springs, and saline lakes? What are the upper temperature limits for efficient functioning of major components of the microbial loop, bacteria, algal picoplankton, protozoa? Further research must address these questions.

Finally, we believe that it is important to remember that the growing evidence for the ubiquity and great importance of the microbial food web in casting the new paradigm does not replace the classical food chain, but includes it. What we have in fact discovered is that the way we have been looking at energy transfers in aquatic systems is no longer comprehensive enough to explain all that we have observed, and thereby we are forced to develop a much more inclusive view:

> Creating a new theory ... is rather like climbing a mountain, gaining new and wider views, discovering unexpected connections between our starting point and its rich environment. But the point from which we started still exists and can be seen, although it appears smaller and forms a tiny part of our broad view gained by mastery of the obstacles on our adventurous way up.
>
> — Einstein and Infeld (1983)

VIII. Epilogue: Some Recommendations

During our literature survey, we were often disturbed to find that the size dimensions of picoplankton as defined by Sieburth et al. (1978) were not well observed. Where filters of 3-μm pore size were used, it was implicit that the upper size limit might extend to <3 μm. But too often the size fraction filtering through a 1-μm pore size filter was regarded as representing picoplankton. We suspect that this apparent negligence may have been due to lack of faith in the size separation achieved by the filtration method used, where filters of 1-μm pore size might actually pull through larger size particles (presumably up to 2 μm diameter) subject to the filtration vacuum used. In dealing with this problem, we believe that it may be more meaningful to standardize the filtration conditions used for securing the picoplankton fraction by naming the type of filter and its pore size (e.g. Nuclepore 1- and 0.2-μm pore size) and fixing the filtration vacuum required (in centimetres of mercury) for obtaining the appropriate size range meeting at least roughly the definition of picoplankton. We opine that the methodology for precise size separation of picoplankton needs to be better investigated, preferably by an international panel of scientists.

As already seen from section IV of this review, the present taxonomic position on picoplanktonic algae, especially the cyanobacteria, leaves much to be desired. Depending on the investigator's background, the chroococcoid cyanobacterium is still occasionally named according to the previous botanical system, while the currently used bacteriological system is not satisfactory to most investigators in the way it offers clone numbers (or symbols) attached to genera such as *Synechococcus* or *Synechocystis*, which, in themselves, appear to be temporary repositories of more tailored genera yet to be defined. It is clear that the entire taxonomy of cyanobacteria is now in a state of flux awaiting revision and ultimate acceptance by future investigators in this area. In the meantime, it may be better to live with clone designations than to hastily assign them names which might later prove to be misleading and erroneus.

Similar taxonomic problems have been hitherto encountered by phycologists in dealing with eucaryotic algae of all sizes, where unfortunately there was a tendency to attach names rather than clone symbols and where considerable confusion ensued subsequent nomenclatural revisions. Hopefully, this may not happen to the eucaryotic picoalgae, whose existence has been just discovered but whose taxonomy has not even been seriously undertaken apart from the few exceptions mentioned in section IV of this review.

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NOTES

Existence de populations uricotéliques chez *Abra alba* et *Dreissena polymorpha* (Bivalves Eulamellibranches)[1]

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Les cellules rénales des bivalves eulamellibranches, *Abra alba* et *Dreissena polymorpha*, peuvent contenir des concrétions d'acide urique, identifiables par méthodes cytochimiques et par spectrométrie d'absorption dans l'ultra-violet. La précipitation d'acide urique et l'abondance des concrétions traduisent un mode d'excrétion de type uricotélique. Celui-ci est indépendant du milieu et caractéristique de certaines populations. L'existence d'un dimorphisme génétique, dans ces espèces, est envisagée à titre d'hypothèse.

The kidney cells of eulamellibranchiate bivalves, *Abra alba* and *Dreissena polymorpha*, may contain uric acid concretions, identifiable through cytochemical methods and ultraviolet absorption spectrometry. The precipitation of uric acid and the large number of concretions indicate a uricolytic excretion method, which is independent of the environment and characteristic of certain populations. A genetic dimorphism may exist.

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Les travaux de Needham (1935, 1938) ont imposé l'idée d'une corrélation entre la nature du produit terminal majeur du catabolisme azoté et la quantité d'eau dont peut disposer un animal. Cette théorie, qui interprète l'uricotélisme comme une adaptation au milieu terrestre, a été remise en question, pour les gastéropodes, dès 1967, par Duerr qui y voit un phénomène phylogénique plutôt qu'adaptatif. Chez les bivalves, au contraire, elle ne semble guère contestée et l'on admet qu'ils sont ammoniotéliques (voir Potts 1967; Bishop *et al.* 1983). Il est vrai que l'excrétion a été peu étudiée dans cette classe et Martin (1983) constate qu'en matière de physiologie rénale, nos connaissances n'ont guère progressé depuis Letellier (1887). Or, ce dernier, après avoir rappelé qu'il existait un désaccord sur la nature des concrétions rénales, conclut qu'elles sont surtout formées de phosphates et nie l'existence des concrétions d'acide urique découvertes par Lacaze-Duthiers (1855). Toutefois, Turchini (1923) décrit à nouveau de telles concrétions chez certains bivalves qu'il considère comme les plus évolués.

Les méthodes actuelles ont confirmé l'existence de concrétions intra et extracellulaires de phosphates (Doyle *et al.* 1978). On sait qu'elles piègent divers métaux en excédent dans le milieu (Ghiretti *et al.* 1972; Bryan 1973; Carmichael *et al.* 1980; George *et al.* 1980) et que leur formation peut être provoquée expérimentalement (Mauri et Orlando 1982). En revanche, on peut estimer, comme Franc (1960), que les techniques utilisées par Turchini (1923) n'ont aucune spécificité et que la présence de concrétions d'acide urique dans le rein des bivalves est douteuse.

Ayant observé, dans certaines populations de *Dreissena polymorpha* et d'*Abra alba*, des concrétions dont les caractères cytochimiques ne pouvaient être attribués à des sels minéraux, nous avons cru intéressant de les soumettre, elles aussi, à des méthodes modernes d'analyse et de rechercher les conditions de leur genèse ou de leur disparition éventuelles.

Matériel et méthodes

Notre étude a porté sur deux Bivalves Eulamellibranches, *Dreissena polymorpha*, dulaquicole, suspensivore, et *Abra alba*, marine, sédimentivore.

[1]Travail exécuté dans le cadre de l'Unité associée n° 680 du C.N.R.S., avec la collaboration de M^me^ S. Bailly pour la spectrométrie d'absorption dans l'ultra-violet.

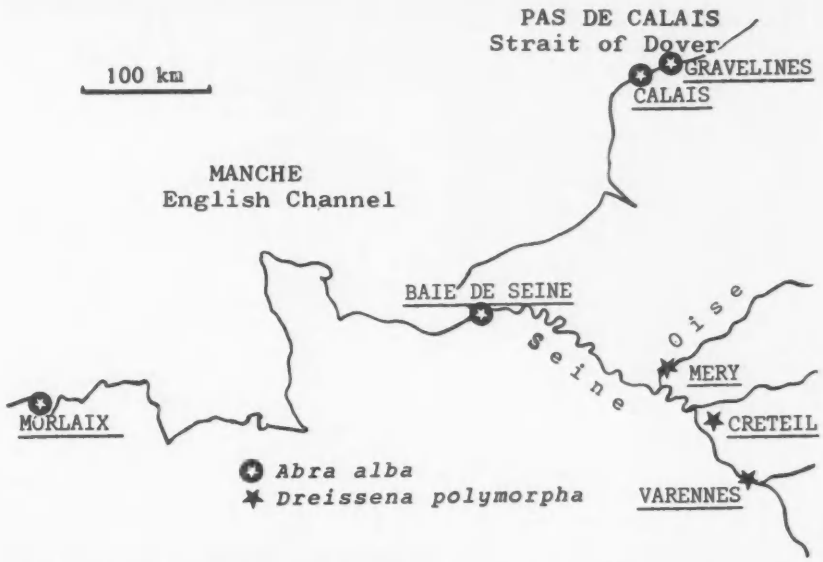


FIG. 1. Origine géographique des populations de *Dreissena polymorpha* et d'*Abra alba* examinées.

Les *Dreissena* ont été ramassées au cours des mois d'avril, d'août, de septembre et de novembre, dans la Seine et dans l'Oise. En Seine, les animaux ont été récoltés sur les parois de l'écluse de Varennes et dans une sablière en eau dite « lac de Créteil », récemment coupée du fleuve et alimentée par une nappe phréatique. Ceux de l'Oise ont été ramassés dans le « bassin de storage » de la compagnie des eaux de Méry, rempli périodiquement par pompage de l'eau de la rivière, mais sans communication permanente avec elle. Quelques individus dragués dans le Léman ont été également examinés. Les *Abra* ont été prélevées en mai, juin, octobre et novembre au large de Calais et de Gravelines, ainsi que dans les baies de Seine et de Morlaix (fig. 1).

Certaines *Dreissena* ont été disposées à raison d'une dizaine par bac de 20 L contenant de l'eau de Seine ou de l'eau minérale naturelle (eau d'Evian), renouvelée tous les deux jours. Elles ont été nourries d'algues lyophilisées du commerce et gardées au laboratoire pendant des périodes allant d'une semaine à un an. Des *Abra* ont été maintenues en aquarium pendant deux semaines. Les autres *Dreissena* et *Abra* ont été fixées dès leur capture ou après quelques heures d'exondation.

Les pièces ont été fixées par les mélanges de Bouin et de Carnoy, incluses et coupées à la paraffine, à l'exception de celles qui étaient destinées à la spectrographie d'absorption dans l'ultra-violet et qui ont été incluses dans l'epon-araldite. L'examen topographique a été mené sur coupes colorées par le rouge solide picro-indigocarmin ou par le trichrome de Prenant-Gabe à l'hématoxyline-éosine-vert solide. D'autre part, nous avons appliqué les méthodes cytochimiques suivantes : coloration au bleu alcian pH 3 (mise en évidence des mucopolysaccharides acides), coloration de Dahl et McGee Russel à la laque d'alizarine et réaction de von Kossa au nitrate d'argent (mise en évidence des sels de calcium), réaction au bleu de Turnbull selon Perls (mise en évidence du fer ferrique), réaction au ferricyanure ferrique selon Schmorl et réaction au complexe d'argentométhénamine selon Gomori (mise en évidence de l'acide urique et des urates). Des préparations témoins ont été soumises à l'action du carbonate de lithium, de l'hydrate de pipérazine, de l'acide acétique et du chloroforme.

Des reins renfermant des concrétions ont été soumis aux trois procédés d'analyse physique suivants.

Spectrographie des rayons X. Des coupes à la paraffine étalées sur mylar carboné ont été analysées par spectrographie dispersive en longueur d'onde au moyen d'une microsonde CAMECA MS 46 (tension d'accélération, 15 kV; intensité de la sonde, 40 nA; diamètre de la sonde, 2 µm; cristaux K.A.P. et P.E.T.).

Analyse élémentaire par émission ionique secondaire. Des coupes à la paraffine étalées sur or poli ont été analysées par une microsonde CAMECA SMI 300 équipée d'un déflecteur électrostatique (bombardement à O_2^+ sous 10 kV et 7,5 µA avec une défocalisation de 30/10; diaphragme de lentille, 200 µm; diaphragme de champ, 600 µm; bande passante de 12 V; convertisseur réglé pour un grandissement de 110 environ). L'analyse spectrale a porté sur l'émission des ions positifs.

Spectrométrie d'absorption dans l'ultra-biolet. Des coupes semi-fines montées dans du glycérol pur (n = 1,46) entre lame et lamelle de quartz (n = 1,46) ont été analysées avec un microspectrophotomètre USMP2 ZEISS dans les longueurs d'onde comprises entre 250 et 350 nm.

Résultats

Rappel morphologique — Le système excréteur des bivalves est un ensemble complexe dont le « rein » n'est qu'un segment assurant la réabsorption des sels et une partie de la sécrétion (voir Pirie et George 1979; Martin 1983). Dans les deux espèces étudiées ici, les cellules rénales sont pourvues d'une grosse vacuole apicale, ce qui, selon Turchini, correspond au type « évolué ». Le structure de ces cellules à vacuole est connue par les descriptions qu'en ont faites notamment Turchini (1923), Carmichael *et al.* (1979), George *et al.* (1980). Elles sont caractérisées par des invaginations basales et des microvillosités apicales, disposition qui rappelle le tube

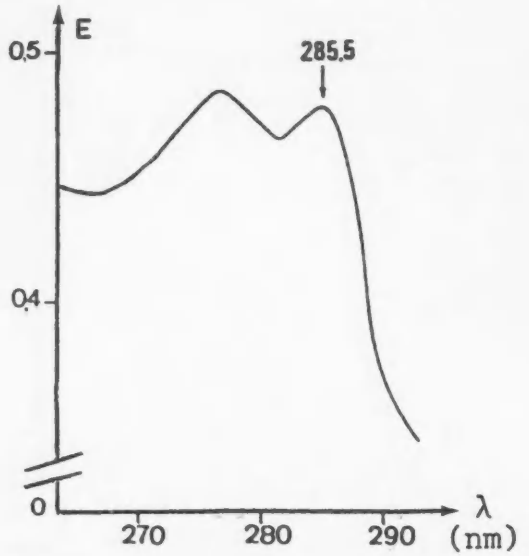


FIG. 2. Courbe d'absorption en ultra-violet des concrétions rénales de *Dreissena polymorpha*. Les longueurs d'onde, exprimées en nanomètres, sont portées en abscisse. Les valeurs d'extinction, exprimées en unités arbitraires, sont portées en ordonnée.

contourné proximal du néphron des Vertébrés. Le noyau et la majeure partie des autres organites sont refoulés à la base par la vacuole apicale. Cette dernière contient ou non des concrétions.

Caractères et nature des concrétions — Les concrétions intravacuolaires se présentent sous plusieurs aspects. Il peut s'agir d'une sphère unique, parfaite et à strates concentriques, ou d'un agglomérat de grains plus petits ou encore d'une sphère entourée d'un halo de matériel pulvérulent. Leur diamètre atteint 10 µm. Leur teinte naturelle va du jaune au brun. Le matériel pulvérulent est biréfringent et les sphères donnent le phénomène de la croix noire. Aucune trame organique n'apparaît par coloration au bleu alcian.

La détection histochimique du Ca et du Fe reste négative. L'analyse par dispersion des rayons X confirme que le Ca (50 coups $\cdot s^{-1}$) et le Fe (15 coups $\cdot s^{-1}$) sont très peu abondants, comme le sont d'ailleurs, le S (10 coups $\cdot s^{-1}$) et le P (6 coups $\cdot s^{-1}$). L'analyse ionique y révèle les éléments Na, Mg, Si, K, Fe, tous en très faible quantité. Au contraire, les réactions de mise en évidence de l'acide urique et de ses sels réagissent positivement. D'autre part, les concrétions qui sont insolubles dans l'eau, l'éthanol, l'acide acétique, le chloroforme et le toluène, sont rapidement dissoutes par le carbonate de lithium saturé et par l'hydrate de pipérazine. Enfin, elles absorbent la lumière ultra-violette à 285 nm, ce qui coïncide avec la zone d'absorption des produits puriques, celle-ci allant de 284 à 290 nm (fig.2).

Ces caractères indiquent que les concrétions sont de nature purique et faiblement minéralisées. Leur insolubilité dans l'eau et leur faible teneur en Na et K permettent de rapporter le composé purique à l'acide urique plutôt qu'à un de ses sels.

Répartition des concrétions — Les concrétions puriques, lorsqu'elles existent chez un individu, se trouvent dans toutes les cellules rénales, à de très rares exceptions près. Leur présence est indépendante du sexe, de l'état de maturité génitale, du cycle saisonnier et de l'âge de l'animal, estimé d'après sa taille. L'infestation par des larves de trématodes est également sans influence. En revanche, tous les individus d'une origine géographique donnée en sont soit pourvus, soit dépourvus. Ainsi, elles existent constamment dans les reins des *Abra* de Gravelines et dans ceux des *Dreissena* de l'Oise. Inversement, elles font toujours défaut chez les *Abra* de Calais, de la baie de Seine et de la baie de Morlaix, ainsi que chez les *Dreissena* du Léman et de la Seine (fig. 3 et 4). Pour ces dernières, il convient de souligner que les deux populations ont certainement une souche commune mais vivent dans deux biotopes différents, l'un étant un lac alimenté par une nappe phréatique et l'autre une écluse ouverte à la navigation.

Les diverses tentatives effectuées pour provoquer la genèse ou la disparition des concrétions, *in vivo*, sont restées inopérantes. Elles n'apparaissent ni chez les *Dreissena* de Seine exondées pendant 48 heures ou gardées en élevage pendant plusieurs semaines, ni chez les *Abra* de Morlaix laissées deux semaines en aquarium. Quant aux *Dreissena* de l'Oise, elles conservent leurs concrétions même lorsqu'elles ont été maintenues durant un an dans des bacs alimentés d'eau de Seine.

Cet ensemble de données suggère que la présence de concrétions puriques n'est pas conditionnée, au moins de manière directe, par la nature du milieu.

Discussion

Les méthodes modernes d'analyse confirment qu'il peut exister, dans le rein des Bivalves, des concrétions puriques comme l'ont affirmé Lacaze-Duthiers (1855) ou Turchini (1923). Ce résultat ne remet pas en question l'existence, dans d'autres espèces, de concrétions de phosphate de calcium également connues depuis le siècle dernier et étudiées par plusieurs auteurs contemporains (voir l'introduction). Il faut donc en conclure que deux types d'inclusions peuvent se trouver dans les cellules rénales des Bivalves, les unes purement minérales, les autres où les composés puriques coexistent avec une faible quantité de sels minéraux. Différentes par leur composition, elles semblent l'être aussi par le mécanisme de leur formation. En effet, alors que les concrétions puriques paraissent très stables en nombre et en taille, dans une population donnée, la genèse et l'importance des concrétions purement minérales sont conditionnées par des facteurs de l'environnement (Doyle *et al.* 1978; Gold *et al.* 1982). Sachant que l'on attribue au rein les deux fonctions de sécrétion et de réabsorption, on peut émettre l'hypothèse que les concrétions puriques se forment à partir des déchets du métabolisme prélevés dans le sang, comme celles de l'Escargot (Bouillon 1960), tandis que les concrétions minérales proviennent de la réabsorption des sels filtrés dans le péricarde.

Les théories de Needham (1935, 1938) selon lesquelles le degré d'uricotélie traduirait une adaptation au milieu terrestre et serait plus élevé dans les formes vivipares, ne s'appliquent pas aux espèces étudiées ici. Il est même assez remarquable que les *Dreissena* de l'écluse de Varennes qui devraient économiser l'eau parce qu'elles sont soumises à des exondations périodiques et parfois prolongées, soient précisément dépourvues de concrétions puriques. Il en va de même en ce qui concerne le développement. Ainsi, *Dreissena*, avec ses larves véligères nageuses, peut être uricotélique alors que le rein de l'espèce incubante *Anodonta cygnaea* ne contient pas d'acide urique (Needham 1935). L'existence de deux populations d'*Abra alba* et de *Dreissena polymorpha* n'est pas sans rappeler le problème

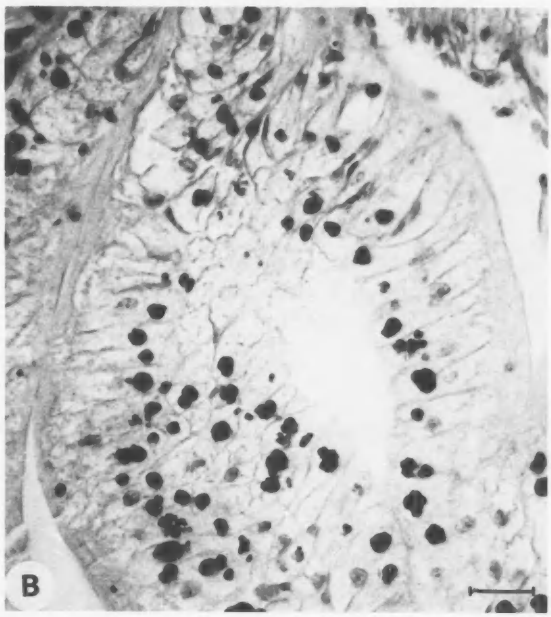
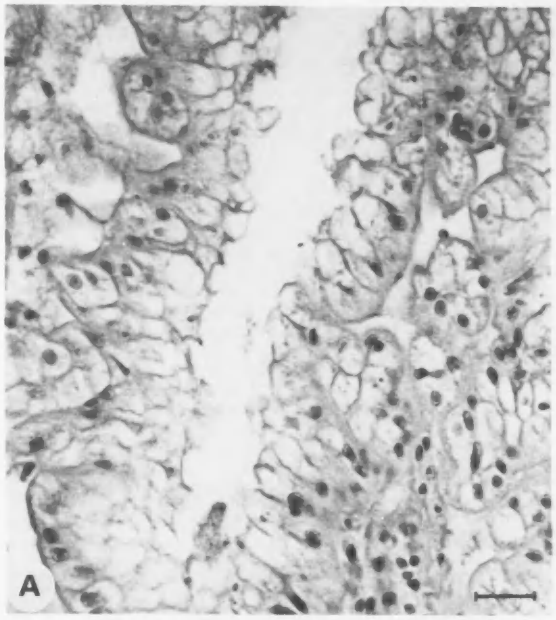


FIG. 3. Aspect histologique du rein d'*Abra alba*. A) Rein d'un animal originaire de Calais, dépourvu de concrétions (fixation par le mélange de Bouin; coloration par le trichrome de Prenant-Gabe; l'échelle représente 60 μm. B) Rein d'un animal originaire de Gravelines, pourvu de concrétions puriques (fixation par le mélange de Carnoy; coloration par le rouge solide–picroindigocarmin; l'échelle représente 25 μm).



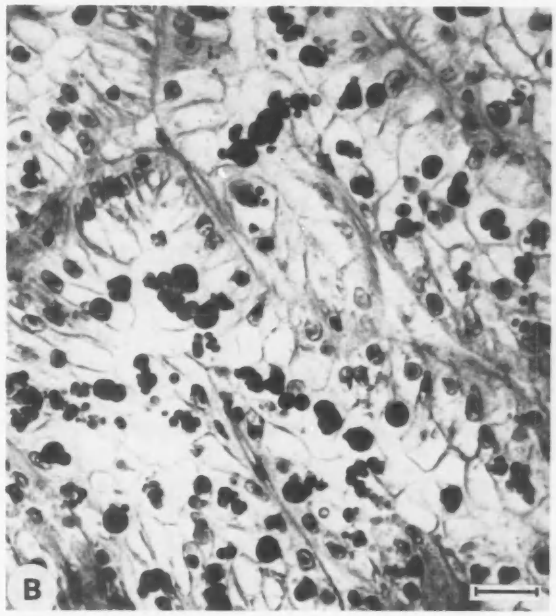


FIG. 4. Aspect histologique du rein de *Dreissena polymorpha*. A) Rein d'un animal provenant de la Seine, dépourvu de concrétions (fixation par le mélange de Carnoy; coloration par le rouge solide–picroindigocarmin; l'échelle représente 20 μm). B) Rein d'un animal provenant de l'Oise, pourvu de concrétions puriques (fixation par le mélange de Carnoy; coloration par le rouge solide–picroindigocarmin; l'échelle représente 15 μm).

des deux espèces de Limnées, *Lymnaea stagnalis* et *L. peregra*, signalé par Needham lui-même, la première détenant 137 $mg \cdot g^{-1}$ dans son rein et la seconde 0,24 $mg \cdot g^{-1}$ seulement dans l'ensemble de ses tissus mous. Dans le cas des bivalves, on ne peut évidemment retenir l'idée que l'une des deux populations aurait colonisé récemment le milieu aquatique et conserverait son mode d'excrétion ancestral.

Bien que les bivalves soient considérés comme ammoniotéliques, un certain uricotélisme y est reconnu puisque l'acide

urique a été identifié et dosé dans le rein lui-même, dans l'ensemble des tissus mous ou dans les excreta de *Pecten*, *Mytilus* et *Unio* (Needham 1935; Spitzer 1937; Ishida 1956). Il s'agit là d'une excrétion d'acide urique sous forme dissoute qui ne représente qu'une fraction minime des déchets du catabolisme purique. Chez *Abra alba* et *Dreissena polymorpha*, la caractérisation de concrétions en place dans le rein prouve qu'il est excrété sous une forme précipitée. Le nombre des concrétions est tel qu'il doit représenter le produit d'excrétion majeur.

L'existence de populations d'une même espèce différant entre elles par leur degré d'uricotélie n'a, à notre connaissance, jamais été signalée. Cette différence, qui suppose pour certaines populations un raccourcissement de la chaîne enzymatique de l'uricolyse, pourrait correspondre à un dimorphisme génétique. Une telle hypothèse concorde avec les données de génétique moléculaire relatives à l'hétérogénéité des populations de bivalves (Badino 1980; Davis *et al.* 1981).

En conclusion, certaines populations d'*Abra alba* et de *Dreissena polymorpha* sont uricotéliques, sans que ce caractère soit imputable à un besoin d'économie d'eau. Morphologiquement, le phénomène se traduit par la présence, dans les cellules rénales, de concrétions d'acide urique, différentes des concrétions de phosphates connues par ailleurs. La coexistence de ces deux modes d'excrétion dans la même espèce pourrait être l'expression d'un dimorphisme génétique.

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Diel Vertical Migrations and Horizontal Distributions of *Limnocalanus macrurus* and *Senecella calanoides* (Copepoda, Calanoida) in Lakes of Southern Ontario in Relation to Planktivorous Fish

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Carter, J. C. H., and K. A. Goudie. 1986. Diel vertical migrations and horizontal distributions of *Limnocalanus macrurus* and *Senecella calanoides* (Copepoda, Calanoida) in lakes of southern Ontario in relation to planktivorous fish. Can. J. Fish. Aquat. Sci. 43: 2508–2514.

Patterns of diel vertical migration of two copepods, *Limnocalanus macrurus* and *Senecella calanoides*, showed marked differences among four lakes in southern Ontario. We hypothesize that diurnal and nocturnal vertical

distributions of these two species reflect their vulnerability to planktivorous fish, which may include alewife (*Alosa pseudoharengus*), lake whitefish (*Coregonus clupeaformis*), lake herring (*Coregonus artedii*), rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), trout-perch (*Percopsis omiscomaycus*), and rainbow trout (*Salmo gairdneri*). Horizontal distributions of the two copepods also differed among four lakes: during early summer both species occurred in much shallower waters in lakes with low transparency than in clearer lakes.

Les modes de migration verticale nycthémérale de deux copépodes, *Limnocalanus macrurus* et *Senecella calanoides* de quatre lacs du sud de l'Ontario présentent des écarts marqués. Les auteurs émettent l'hypothèse que les distributions verticales diurnes et nocturnes de ces deux espèces reflètent leur vulnérabilité aux poissons planctivores, à savoir le gaspareau, le gaspareau (*Alosa pseudoharengus*), le corégone de lac (*Coregonus clupeaformis*), le cisco (*Coregonus artedii*), l'éperlan (*Osmerus mordax*), la perchaude (*Perca flavescens*), l'omisco (*Percopsis omiscomaycus*) et la truite arc-en-ciel (*Salmo gairdneri*). Les distributions horizontales de ces deux copépodes diffèrent aussi entre les quatre lacs : au début de l'été, les deux espèces se trouvaient dans des eaux beaucoup moins profondes dans les lacs à eaux moins transparentes.

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The calanoid copepods *Limnocalanus macrurus* Sars and *Senecella calanoides* Juday are glacial relicts and, in northeastern North America, are confined to areas formerly occupied by glacial lakes or their spillways (Dadswell 1974; Carter et al. 1980). Because they are usually observed in the hypolimnia of deeper lakes, they are considered cold stenotherms. While this is undoubtedly true, it is also probable that neither of these large species would be able to survive in well-lit epilimnia under pressure from size-selective fish predators. *Limnocalanus* is known to undergo extensive diel vertical migrations (Wilson and Roff 1973); previous studies on migration of *Senecella* have been inconclusive because of small samples.

At least 50 lakes in southern Ontario contain *Limnocalanus* and/or *Senecella*. We investigated patterns of diel vertical migration in four lakes and of horizontal distribution in four lakes. We found striking between-lake differences in the patterns of distribution of both species, and we considered the possibility that these might be reflections of predation pressure. This note examines the hypothesis that diurnal and nocturnal locations of *Limnocalanus* and *Senecella* populations are closely related to the visually seeking fish planktivores present in each lake.

Methods

The seven lakes of the survey are all located in the northern part of southern Ontario (Fig. 1). Horizontal distributions were studied in June–July and September in each of Gull, Turtle, Manitouwabing, and Wauquimakog during 1980. Diel vertical migrations were investigated over a single 2-d period in each of Charleston, Gull, Smith, and Weslemkoon during 1981.

Vertical distributions in all lakes were obtained by a single sampling of contiguous strata from bottom to surface with a conical closing plankton net (Carter 1969) of 0.5 m mouth diameter and No. 10 mesh (apertures 153 μm). Each series was begun with the net resting on the lake bottom. Waters within 40 m of the surface were sampled over 4- or 5-m intervals and waters below 40 m over 10-m intervals. The net was hauled from an anchored boat at a rate of approximately 0.5 $m \cdot s^{-1}$. The filtering efficiency of the net was assumed to be 100%. Evans and Sell (1985) found nets of identical mouth diameter and mesh size to be 98% efficient.

Horizontal distributions were defined by determining midday vertical distributions at either three or four locations within the basin of greatest depth. Bathymetric maps obtained from Ontario Ministry of Natural Resources (MNR) were used to select station sites. The first station approximated the lakes maximum sounded depth. All subsequent stations were of decreasing depth with the shallowest being not less than 7 m deep. Location of the shallower stations was determined by lake morphometry, with regions of gradually decreasing depth being preferred. Consequently, stations did not lie along a true transect, although an attempt was made to position stations along as straight a line as possible.

All diel vertical migration series were conducted at a single deepwater station in each lake. In most cases, four vertical series were collected over the 2-d period: during full daylight, near sunset, during full darkness, and near sunrise. In Charleston the sunrise series was omitted. For each migration study, the phase of the moon was between the last quarter and new moon, and the nocturnal samples were collected before moon rise. Because we frequently left the station after completing a series of hauls, we were often unable to maintain the exact depth throughout each study. Each series required 1–2 h to complete, depending on depth. In the laboratory all individuals of both species were counted under a Wild M8 dissecting microscope.

The difficulties involved in interpreting migration data from samples collected sequentially and at relatively wide time intervals between series have been fully discussed by Pearre (1979). Unfortunately there is no easy solution to these problems. The plotted vertical distributions were usually found to be unimodal and were therefore accepted as reasonable approximations to reality. The term population displacement, used throughout, is defined as the perceived change in position of the population mean depth.

A temperature profile was taken at the deepest station in each lake with a Whitney Underwater Thermistor (model TC-5A) equipped with a 60-m cable. The epilimnion, metalimnion, and hypolimnion were later delimited graphically using Hutchinson's (1957) definition. Water transparency was measured with a 20-cm-diameter black and white Secchi disc and apparent color with a BDH Lovibond Nesslerizer (model MK3). Morphometric parameters are either from Dadswell (1974) or derived from his unpublished bathymetric maps. Total calcium–magnesium hardness is also taken from Dadswell. Fish species lists for each lake, based on an intensive survey during 1970–72, were obtained from the MNR regional offices in Brockville, Minden, North Bay, Parry Sound, and Tweed, Ontario.

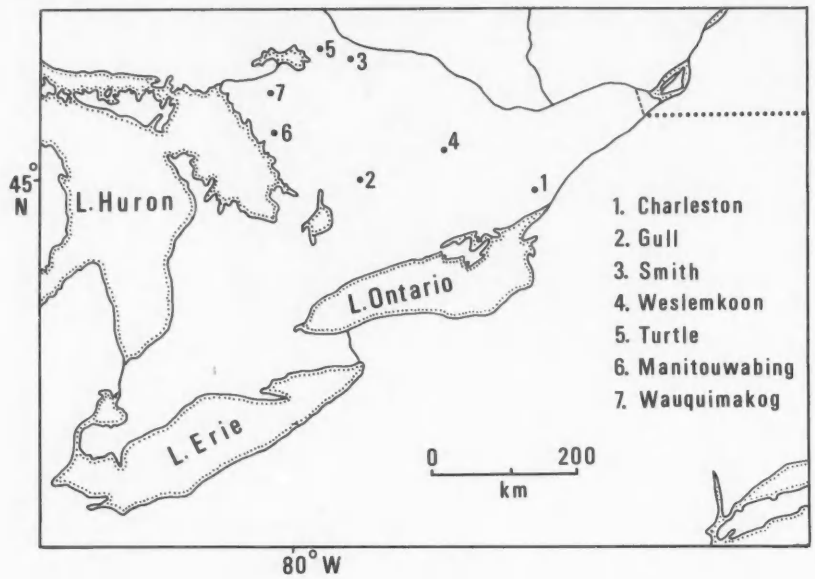

FIG. 1. Southern Ontario showing study lake locations.

TABLE 1. Morphometric, chemical, and physical characteristics of the seven lakes of the study. SD = Secchi depth; TH = total hardness.

Lake	Area (ha)	Z_{max} (m)	Z_{mean} (m)	Color (Hazen units)	SD (m)	TH (ppm)
Charleston	2490	92	17.1	10	5.7	130
Gull	996	39	16.2	5	5.8	30
Smith	700	28	—	30	3.6	—
Weslemkoon	1933	55	9.1	10	6.7	18
Turtle	210	50	—	15	3.7	30
Manitouwabing	1235	24	5.5	45	2.7	25
Wauquimakog	470	29	10.3	35	2.7	25

Lake characteristics — The lakes range in area from 210 to 2490 ha (Table 1). However, some of the lakes (e.g. Weslemkoon, Charleston) are multibasined so that total area is not always a good indicator of open-water expanse. Maximum depths range from 28 to 92 m. Smith, Wauquimakog, and Manitouwabing are fairly dystrophic with color ≥30 Hazen units. These three and Turtle also had relatively low Secchi transparencies. Most of the lakes are located entirely on the Precambrian Shield and hence contain comparatively soft water. Charleston borders on the St. Lawrence Lowlands and has a total hardness of 130 ppm.

Both copepod species have been recorded in five of the seven lakes. However, in Wauquimakog the population of *Senecella* is so small that is is not considered in this study. Weslemkoon contains only *Limnocalanus* and Manitouwabing only *Senecella*.

Results

Diel vertical migrations — In Charleston in late August, C5 instars of *Senecella* underwent an extensive vertical migration (Fig. 2) with a maximum population displacement of 40.4 m (Table 2). At sunset (21:15), the time of highest observed ascent, part of the population had penetrated the metalimnion. By midafternoon (14:30), mean depth was 61.0 m; a few animals were in the deepest waters but the great majority occupied the 50–70 m stratum. *Limnocalanus* in Charleston carried out a comparatively minor vertical migration with a maximum population displacement of 11.7 m. Even at sunset, the period of observed maximum ascent, very few individuals were above 35 m. By midafternoon, most *Limnocalanus* were located in the deepest waters, with a mean depth of 73.4 m, 12.4 m below that of *Senecella*.

Distributions of *Senecella* and *Limnocalanus* in Gull largely overlapped at all times (Fig. 2). About half the animals of both species appeared to migrate while the remainder stayed in the deepest 5 m. Maximum population displacement was only 4.7 m for *Senecella* and 5.2 m for *Limnocalanus* (Table 2) although these figures are complicated by the greater depth sampled at 01:45. By midday both species were located almost entirely in the deepest stratum; numbers at that time were very low, suggesting that many animals might have been close to the sediment and hence escaped capture.

In Smith, both species appeared to undergo a distinct migration although sampling error was undoubtedly magnified in the small *Senecella* population (Fig. 2). At 15:30, mean depth of *Limnocalanus* was 22.6 m (Table 2). By 02:45, the time of

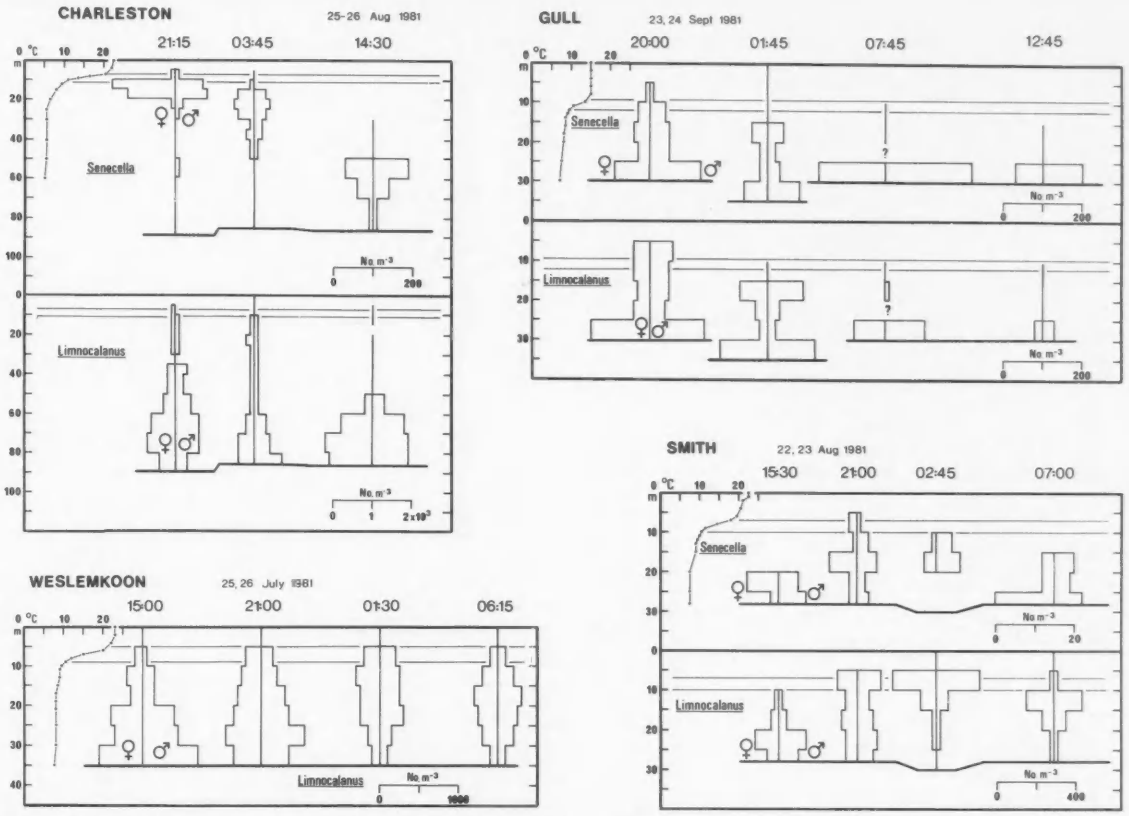

FIG. 2. Diel vertical migrations of *Limnocalanus* and *Senecella* in Charleston, Gull, Smith, and Weslemkoon lakes. Times indicate approximate midpoint of each series. Thin horizontal lines delimit the epilimnion, metalimnion, and hypolimnion. Heavy horizontal lines indicate the bottom. Sample from Gull from 20–25 m at 07:45 accidentally destroyed.

TABLE 2. Mean depth of distribution (m) at midday (D), sunset (S), and night (N) and maximum observed population displacement (PD) of *Limnocalanus macrurus* (adults) and *Senecella calanoides* (copepodite 5) in four southern Ontario lakes.

	Limnocalanus				*Senecella*			
Lake	D	S	N	PD	D	S	N	PD
Charleston[a]	73.4	61.7	63.2	11.7	61.0	20.6	28.8	40.4
Gull[a]	29.3	24.1	26.1	5.2	29.8	25.1	26.2	4.7
Smith[a]	22.6	16.8	9.8	12.8	24.6	18.2	15.7	8.9
Weslemkoon	25.0	20.0	18.2	6.8				

[a]Mean depths adjusted to compensate for between-series variations in bottom depths.

observed maximum ascent, *Limnocalanus* mean depth was 9.8 m with the mode of distribution in the metalimnion or above.

Distribution of *Limnocalanus* in Weslemkoon at 15:00 was triangular in shape with the base at the lake bottom and the apex, representing a density of about $200 \cdot m^{-3}$ in the 5–10 m stratum (Fig. 2). By 01:30, most animals had left the deeper strata with highest numbers between 5 and 25 m. Population displacement over this 10.5-h period was 6.8 m (Table 2).

Horizontal distributions — In Gull, *Senecella* and *Limnocalanus* were almost entirely restricted to the central deeper region during both July and September (Fig. 3). A few individuals were found at 30 m, but both species were absent at the two shallower stations (22 and 10 m). Similarly, in Turtle, few animals occurred where the depth was ≤25 m. Distribution of *Senecella* in Manitouwabing extended into fairly shallow water (15 m) in late June, but by early September most animals were situated well below the metalimnion with only two individuals at the 12-m station. *Limnocalanus* in Wauquimakog occurred almost to the surface in late June and was abundant even at the 8-m station. By mid-September virtually all animals were confined to the two deeper stations (22 and 32 m) and none were found at depths <12 m.

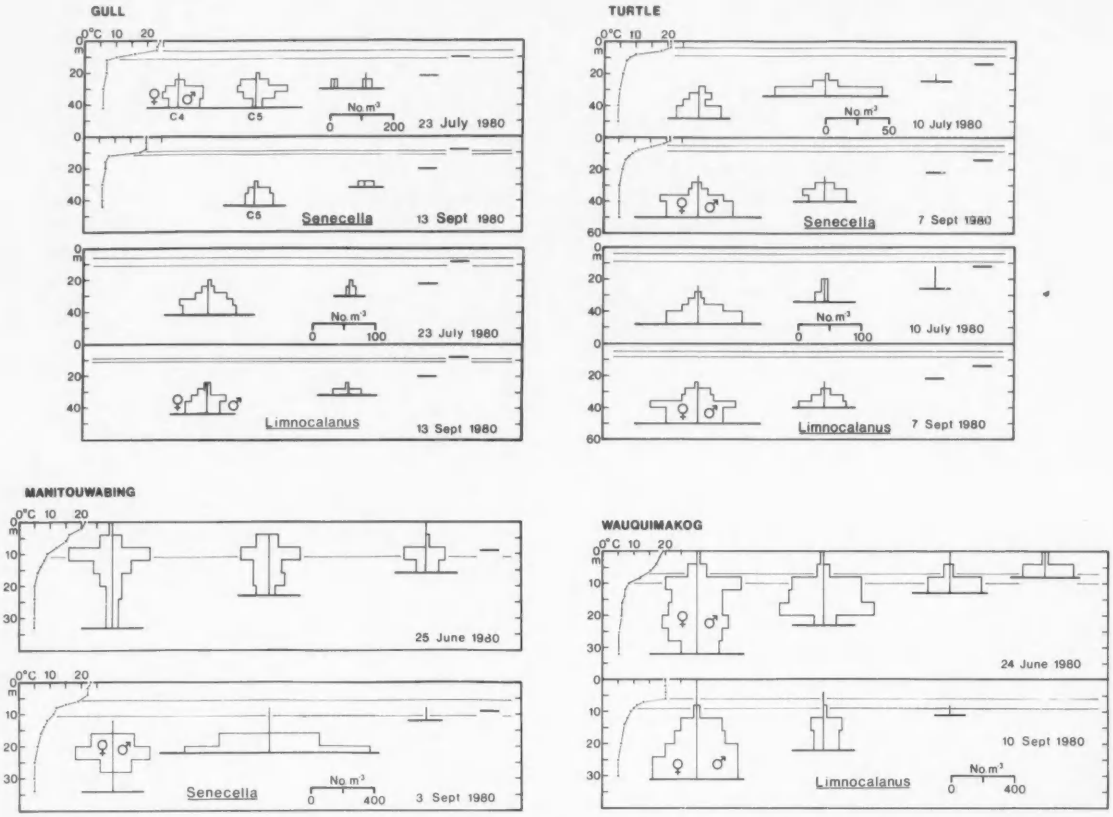

FIG. 3. Horizontal distributions of *Limnocalanus* and *Senecella* in Gull, Turtle, Manitouwabing, and Wauquimakog lakes. Thin horizontal lines delimit the epilimnion, metalimnion, and hypolimnion. Heavy horizontal lines indicate the bottom.

Discussion

While water transparency is usually positively related to both extent of vertical migration and daytime depth of zooplankton (Hutchinson 1967), differences in population displacement between lakes of this study were either unrelated to transparency (*Senecella*) or showed an apparent negative relationship (*Limnocalanus*) (Fig. 4). Moreover, the migration patterns of each species relative to the other were often reversed between lakes, e.g. in Charleston, population displacement of *Senecella* was almost four times that of *Limnocalanus*, but in Smith only about half as great (Table 2). Neither does water temperature appear to have been critical, at least for many animals, e.g. in Charleston, few *Limnocalanus* ever experienced water warmer than 5°C, while in the other three lakes most individuals of this species were always at temperatures somewhat (and often considerably) higher.

The influence of fish predation on patterns of zooplankton diel migration has been well documented (e.g. Zaret and Suffern 1976; Stich and Lampert 1981). *Limnocalanus* and *Senecella* adults are between 2 and 3 mm in body length and therefore must be extremely vulnerable to size-selective predators (Zaret 1980). It can therefore be speculated that the often extensive diel migrations of these two species have evolved in response to predation pressure.

Charleston shows two extremes of migratory behaviour that are unlike those in the other four lakes. *Senecella* underwent a dramatic nocturnal ascent from a mean depth of 61.0 m in daytime to a mean of 20.6 m at sunset, while *Limnocalanus* occupied the deepest waters during the day and made only a modest rise at night (Fig. 2; Table 2). Postulating that these two divergent patterns may be related to fish planktivory, then Charleston might be expected to exhibit very high predation pressure.

Table 3 lists seven important fish species which are planktivorous during at least part of their life cycle and which occur in the open waters of one or more of the lakes. Charleston has only four of these species, but most notably it is the only lake which contains the alewife, *Alosa pseudoharengus*, a voracious and obligate planktivore. Although the history of the alewife in Charleston is not known for certain, it probably dates back at least 12 000 yr to the period when the lake was within the western boundary of the Champlain Sea (Prest 1970). It is apparently quite abundant, as periodic die-offs have been observed (M. L. Hart, Ontario MNR, Brockville, Ont. K6V 5V8, pers. comm.). The effectiveness of the alewife in reducing or eliminating large zooplankters was first documented by Brooks and Dodson (1965). But even earlier, *Limnocalanus* was noted in stomachs of alewife from the lower Great Lakes (Pritchard 1929; Price 1963). Morsell and Norden (1968) found that *Limnocalanus* comprised a large part of the alewife diet in the profundal zone of Lake Michigan, particularly in winter. Wells

TABLE 3. Major species of planktivorous fish occurring in each of the eight lakes of the study (data from Ontario Ministry of Natural Resources).

Species	Charleston	Gull	Smith	Weslemkoon	Turtle	Manitouwabing	Wauquimakog
Alosa pseudoharengus	+	−	−	−	−	−	−
Coregonus artedii	+	+	+	−	+	+	+
Caregonus clupeaformis	+	+	+	−	−	+	+
Osmerus mordax	−	+	−	−	−	−	−
Perca flavescens	+	+	+	+	+	+	+
Percopsis omiscomaycus	−	+	−	−	−	−	−
Salmo gairdneri	−	−	+	−	−	−	−

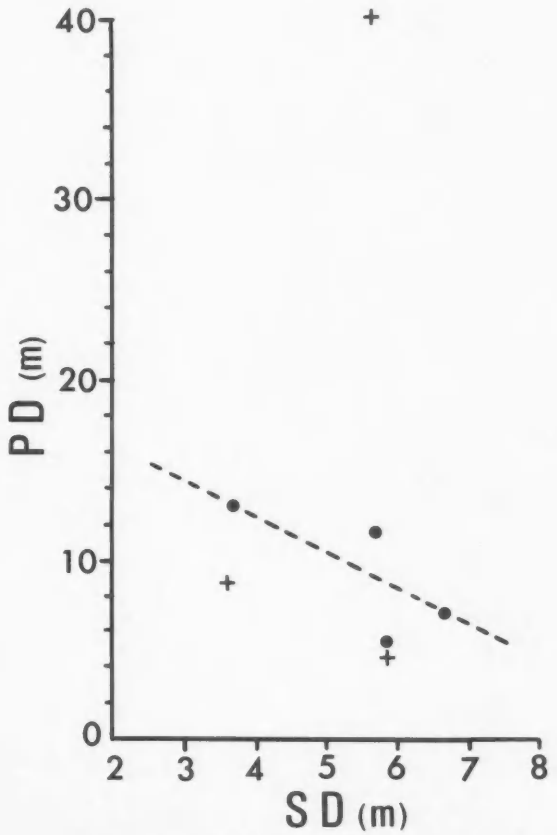

FIG. 4. Relationship between Secchi depth (SD) and maximum observed population displacement (PD) of *Limnocalanus* (●) and *Senecella* (+) in four lakes. Broken line fitted by eye to *Limnocalanus* points only.

(1970) described a dramatic decline in *Limnocalanus* in the same lake in conjunction with an enormous increase in alewife numbers; *Senecella* also declined, although numbers were too small to allow firm estimates.

Although alewife may occur at all depths (Argyle 1982), greatest abundances are usually found at temperatures >8°C (Wells 1968). This may account for the fact that *Limnocalanus* remains in the cold waters of the middle and lower hypolimnion in Charleston at all times (Fig. 2). As it is known to feed intensively on smaller copepods (Bowers and Warren 1977; Warren 1983), permanent exclusion from the phytoplankton zone may not be crucial. Similar nonmigratory behavior by deepwater copepods where predation is high has been documented for *Cyclops abyssorum tatricus* in eastern Europe (Gliwicz and Rowan 1984).

Residing above *Limnocalanus* in the middle hypolimnion during the day, *Senecella* ascends to the base of the metalimnion, or slightly higher, at night (Fig. 2). *Senecella* is an extremely fast swimmer (Ramcharan 1985) and this may enable it to avoid capture by planktivores whose feeding efficiency is reduced in darkness. It is also reported as more herbivorous than *Limnocalanus* (Wong and Chow-Fraser 1985) and may be obliged to graze in the upper waters at night.

We have no information regarding densities of the other six planktivores in the lakes. The lake herring, *Coregonus artedii*, is known to feed on copepods (Dryer and Beil 1964; Janssen 1978). Lake whitefish, *C. clupeaformis*, are also planktivorous (Kliever 1970) and both coregonids could be expected to occupy the middle and deeper waters of large lakes. Rainbow smelt, *Osmerus mordax*, feed on *Limnocalanus* and have been suggested as contributing to its decline in Lake Erie (Gannon and Beeton 1971). Yellow perch, *Perca flavescens*, are planktivorous as juveniles (Noble 1975) but are probably restricted mainly to the upper and inshore waters. Trout-perch, *Percopsis omiscomaycus*, can be planktivorous and may also enter deeper waters (Dadswell 1972). The feeding of rainbow trout, *Salmo gairdneri*, on zooplankton has been frequently documented (Ware 1972; Taylor and Gerring 1985), although they seem to more often prefer larger food items such as small fish (Larkin and Smith 1954; Fraser 1978).

The effects of planktivory on the migratory behavior of *Limnocalanus* and *Senecella* in the other three lakes are more difficult to determine. In Gull, both species concentrated in the deepest waters at midday (>25 m; Fig. 2) which may relate to the presence of all major planktivores except alewife and rainbow trout; at night the upward displacement of both copepod populations was relatively minor (Table 2). Smith lacks smelt and trout-perch, although it does have a stocked population of rainbow trout; here, both *Limnocalanus* and *Senecella* were located considerably higher in the water column at most times than they were in Gull (Fig. 2; Table 2). Possibly, rainbow trout actually reduce planktivory in Smith by predating the smaller planktivores. *Limnocalanus* in Weslemkoon made a general descent into deep water during the day, but a sizeable proportion of this large population remained in the upper waters at all times (Fig. 2); this lake lacks all planktivores except yellow perch.

Horizontal distribution should reflect predation from specifically inshore planktivores in addition to the fishes already considered. These would include numerous small cyprinids (shiners, minnows, and chubs), many species of which may be

found in these lakes. In Gull and Turtle (Fig. 3), both copepods were rare or absent in waters less than 30 m deep during both July and September. On the other hand, in late June, *Senecella* in Manitouwabing and *Limnocalanus* in Wauquimakog were both common in shallow inshore waters. These two lakes are more highly colored and with lower Secchi transparency than Gull and Turtle (Table 1) and the reactive distance of visually seeking planktivores may be reduced accordingly (Kitchell and Kitchell 1980; Carter et al. 1983). This protection is apparently limited, since by early September both species were almost totally confined to waters of greater depth. Whether this resulted from actual physical elimination in the shallows or from horizontal migration into deeper waters is unknown.

Acknowledgements

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Sequential Tests for Infectious Hematopoietic Necrosis Virus in Individuals and Populations of Sockeye Salmon (*Oncorhynchus nerka*)

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Mulcahy, D. M., and R. J. Pascho. 1986. Sequential tests for infectious hematopoietic necrosis virus in individuals and populations of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 43: 2515–2519.

The incidence and titer distribution of infectious hematopoietic necrosis virus in cavity fluid from spent female sockeye salmon (*Oncorhynchus nerka*) varied little when fish from a naturally spawning population were sampled three times on alternate days. However, when prespawning female sockeye salmon from a second population were individually tagged, penned, and sampled daily, the incidence and proportion of fish with high virus titer rose over a 6-d period. In 10 instances, consecutive cavity fluid samples from the same fish reverted from virus-positive to virus-negative. We suggest that spent fish should be sampled when accurate and quantitative data on the incidence and level of the virus are required.

La fréquence et la distribution des concentrations du virus responsable de la nécrose hématopoïétique infectieuse dans le liquide de la cavité abdominale provenant de saumons sockeye (*Oncorhynchus nerka*) femelles vidés de leurs oeufs ont peu varié lorsqu'on a échantillonné à trois reprises, un jour sur deux, des poissons provenant d'une population qui se reproduit naturellement. Cependant, lorsqu'on a étiqueté individuellement des saumons sockeye femelles n'ayant pas encore frayé provenant d'une deuxième population, qu'on les a mis en enclos et échantillonnés quotidiennement, la fréquence et la proportion de poissons ayant une forte concentration en virus ont augmenté sur une période de 6 jours. Dans dix cas, des échantillons consécutifs de liquide de la cavité abdominale prélevés chez le même poisson sont passés de positifs à négatifs en ce qui concerne la présence du virus. Nous proposons que les poissons vidés de leurs oeufs soit échantillonnés lorsque des données précises et quantitatives sur la fréquence et la concentration du virus sont nécessaires.

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Infectious hematopoietic necrosis (IHN) is an acute rhabdoviral disease of salmonid fishes characterized by a putative lifelong latent infection with virus production only at the extremes of the host's life cycle. The appearance of the virus in fish as they spawn would permit its vertical transmission (Mulcahy and Pascho 1985). Efforts to control losses of hatchery fish due to IHN depend on the testing of populations by examination of spawning adults to detect the virus and ensure the use of virus-free eggs for production of fish. Existing protocols describe acceptable laboratory procedures for the examination of samples for the detection of IHN virus (Department of Fisheries and Oceans 1984; Amos 1985). Cavity ("ovarian") fluid (CF), the clear viscous liquid associated with the eggs of a ripe fish, is the only acceptable type of sample from an adult fish. For convenience, samples are typically taken on a single day during the spawning season. The incidence and level of IHN virus is related to the spawning condition (ripeness) of the fish; only a small proportion of prespawning (green) fish shed virus at even low levels (Mulcahy et al. 1982). Virus incidence and shedding in spent fish is highest at the end of the spawning season. Virus incidence and levels determined by traditional sampling protocols might not be accurate if individual fish and fish populations are examined only once. The results of testing fish for IHN virus might be strongly influenced by the viral status of the individual fish and that of the population being sampled on a given day.

The purpose of this study was to use the understanding of IHN virus kinetics in sockeye salmon (*Oncorhynchus nerka*) populations gained in previous studies to illustrate that the use of naturally spent fish for quantitative virus examination of fish populations gives stable results. Further, by examining sequential samples from the same fish, we showed that the results obtained from use of ripe fish, the usual source of samples from hatchery fish, are altered by the daily fluctuations in virus incidence and titer.

Materials and Methods

Fish and samples — To determine the variation obtained by sampling spent fish from a population on different days, we used 1978 brood year sockeye salmon from the Lower Thumb River, a tributary of Karluk Lake, Kodiak Island, Alaska. Fish were collected with a beach seine, and we selected only spent fish, which were easily identified by their slack abdomens and the expression of few eggs when stripped. Because Pacific salmon die soon after spawning and IHN virus expression is related to spawning condition, the sampling of naturally spent fish from wild fish populations offered a degree of synchrony

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TABLE 1. Tests on cavity fluid from naturally spent Lower Thumb River sockeye salmon for IHN virus. All fish had spawned naturally. Minimum level of virus detected on each of the three sample days was 10 pfu/mL.

Day	N	Percent positive	IHN virus titer (pfu/mL) Mean	SE	Maximum	Percent of titers $>10^5$ pfu/mL
1	39	62	2.5×10^3	1.7×10^1	6.2×10^7	25
3	52	65	3.0×10^3	3.4×10^1	3.2×10^7	30
5	45	64	8.5×10^3	1.9×10^1	1.5×10^7	34

and stability. Cavity fluid was obtained by extruding a small amount of eggs into a cup and decanting the fluid. The adipose fin was clipped to prevent resampling and the fish was returned to the river. The CF samples were iced, flown to Seattle, and held frozen at −76°C until processed. Fish from this population were sampled three times, on alternate days.

The 1980 brood year sockeye salmon population spawning in the Cedar River, a tributary to Lake Washington in the State of Washington, was used for the study of daily variation of virus incidence and level in individual fish. The Cedar River population has had an IHN virus incidence greater than 92% every year since 1979 (Mulcahy et al. 1983b). A total of 58 females in prespawning condition, recognized by the lack of expression of eggs or CF from the genital opening when firm pressure was applied to the abdomen, were selected from fish trapped for use as hatchery brood stock. Each fish was identified with a numbered filament tag placed in the musculature immediately below the dorsal fin, and held in a wooden pen placed in a side channel of the Cedar River that was used to hold other fish for hatchery use. Beginning the next day, and for the next 6 d, a CF sample was collected from the fish that had ripened. The fluid was decanted and held at 4°C until processed the next day.

We detected and quantified IHN virus by the plaque assay (Burke and Mulcahy 1980), using the epithelioma papulosum cyprini (EPC) cell line (Fijan et al. 1983). The CF was added to cell monolayers undiluted and as serial $\log_{10}$ dilutions to 10^{-5}. Viral titers were calculated as plaque-forming units per millilitre (pfu/mL). Mean virus titers were calculated after transformation of individual titers to $\log_{10}$ values. We constructed histograms of the distributions of daily virus titers on the basis of $\log_{10}$ pfu/mL classes. The number of samples with titers in each class were expressed as a percent of the total number of virus-positive samples for that day, to compensate for the different numbers of positive samples obtained each day.

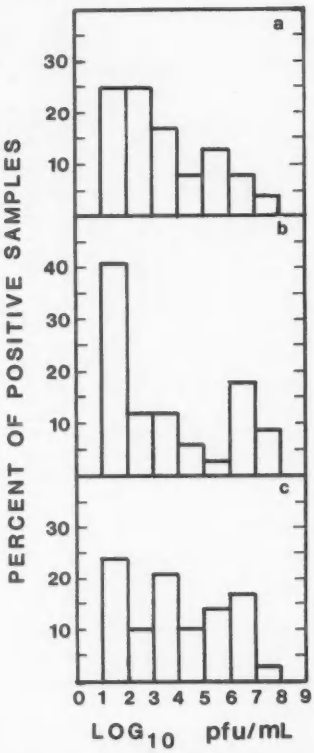

FIG. 1. Frequency distribution of daily IHN virus titers in cavity fluid from naturally spent female sockeye salmon spawning in the Lower Thumb River and sampled on alternative days (a, b, c). See Table 1 for details.

Results

Variation in expression of IHN virus in spent fish — Repeated sampling of CF from naturally spent female sockeye salmon from the Lower Thumb River on alternative days resulted in population infection rates (62−65%) and mean virus titers (2.5×10^3 to 8.5×10^3 pfu/mL) that varied little (Table 1). There was a wide range of individual virus titers (about 7 $\log_{10}$), but that range changed little between sampling dates (Table 1). The distribution of individual virus titers on each of the sampling dates was similar (Fig. 1), except that the number of fish with very low titers increased on the second sampling day. There was a slight increase, from 25 to 34%, in the proportion of fish with high levels ($>10^5$ pfu/mL) of virus, indicating that release of IHN virus by fish of this population had not peaked.

Daily variation in IHN virus expression in fish in spawning condition — In most examinations of hatchery and wild salmonid fish populations for IHN virus, CF from ripe fish has been used. In ripe fish, IHN virus may not be at its fullest development. Holding tagged fish from the Cedar River and sampling each fish daily enabled us to follow the changes in IHN virus production in individual fish at the stage of spawning condition usually tested and to examine the influence of the maturity of virus production in individual fish on the statistics (percent incidence, mean titer, titer distribution) of virus status for the population. All of the 48 fish in this experiment were initially in prespawning condition, but 43 ripened overnight. The IHN

TABLE 2. Viral incidence and levels obtained from daily viral testing of 48 tagged (reduced to 47 on days 5 and 6 due to the death of one fish) individual female Cedar River sockeye salmon held in a pen. All fish were in prespawning condition the day before the first viral sample was taken. Fish not sampled were in prespawning condition.

Day	N	Percent positive	IHN virus titer (pfu/mL)				Percent of titers $>10^5$ pfu/mL
			Mean	SE	Maximum	Maximum	
1	43	72	1.1×10^4	4.1×10^1	1.0×10^1	3.0×10^8	35
2	47	89	2.2×10^4	2.7×10^1	1.0×10^1	1.9×10^8	35
3	47	90	2.2×10^4	4.2×10^1	1.0×10^1	3.1×10^8	54
4	48	96	3.0×10^5	2.4×10^1	1.0×10^1	3.0×10^8	55
5	47	100	1.8×10^6	1.1×10^1	4.6×10^2	3.0×10^8	62
6	47	98	4.1×10^6	1.1×10^1	3.3×10^2	3.0×10^8	76

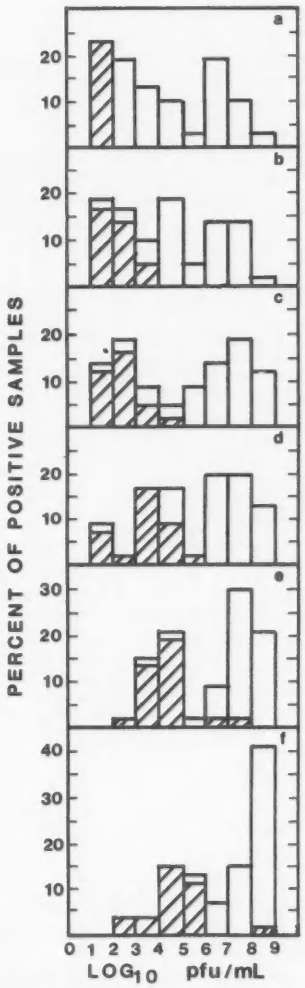

FIG. 2. Frequency distribution of daily IHN virus titers in cavity fluid from Cedar River female sockeye salmon selected for prespawning condition the day before the beginning of the experiment. All fish were tagged and sampled daily for six consecutive days (a, b, c, d, e, f). Shading indicates samples from fish that were in prespawning condition on the first day or those whose first sample was negative. See Table 2 for additional details.

virus infection rate in this group of fish was 72% on the first sampling day and increased to 100% by the fifth sampling day (Table 2). The daily mean virus titers also increased steadily by 373% over the course of the experiment and the range of individual virus titers decreased from 8 to 6 $\log_{10}$ (Table 2). From the increases in infection rate and mean virus titer, and the decrease in the range of virus titers over the sampling period, we concluded that development of IHN virus infections in this experimental group of fish had not yet peaked. The distribution of virus titers for each day corroborated this conclusion, showing a steady trend toward high levels of IHN virus in most fish (Fig. 2). This trend could be expressed figuratively in the daily proportion of high ($>10^5$ pfu/mL) titers, which increased from 35% on the first day to 76% on the sixth day (Table 2).

On the first sample day, 5 fish remained in prespawning condition, and 12 fish gave CF that was apparently free of IHN virus (Table 3). These fish are of particular interest from the standpoint of examinations of broodstocks or of the screening of populations for IHN virus. For example, the five fish in prespawning condition would not have been sampled using accepted IHN sampling protocols. Yet, these fish were all positive for virus only 24 h later, and eventually all of them yielded titers exceeding 10^7 pfu/mL. Even more interesting were the 12 fish with initially negative CF samples that later yielded positive CF samples. The use of these fish as part of a population survey or broodstock examination would have affected the accuracy of the population statistics being estimated.

Only 2 of the 19 fish that were initially negative for virus or that had titers of between 10^1 and 10^2 pfu/mL developed extremely high titers (Fig. 2). The fish that were initially negative for virus or with virus titers of less than 10^2 pfu/mL on the final sampling day represented 100% of the samples with titers less than 10^5 pfu/mL but only 17% of the samples with titers greater than 10^5 pfu/mL.

On 10 occasions, sequential samples from fish reverted from positive on one day to negative on the next. Most of these reversions occurred with samples from fish initially negative for virus, or from fish with very low initial virus titers, and may have reflected the threshold of sensitivity of the virus detection system. However, one fish (Fish 4, Table 3) that was negative on the first sampling day reverted from positive to negative twice on succeeding sampling days, including a decrease from 4.7×10^4 pfu/mL on day 5 to negative on day 6.

Discussion

In examining animal populations for the presence of a virus,

TABLE 3. Daily levels of IHN virus in cavity fluid of tagged female Cedar River sockeye salmon that were negative or in prespawning condition on the first day of sampling. These fish are part of the experiment summarized in Table 2. Virus titers were transformed to $\log_{10}$ values. ND = not done; PS = prespawning conditions (no cavity fluid produced).

Fish No.	IHN virus titer (pfu/mL) on day:					
	1	2	3	4	5	6
1	0	2.74	4.48	4.56	6.48	8.04
2	0	3.52	2.41	3.59	4.30	3.91
3	0	1.54	0	4.04	3.32	4.26
4	0	1.30	0	3.97	4.67	0
5	0	2.30	0	1.00	3.49	4.23
6	0	2.32	3.67	3.71	Dead	Dead
7	0	0	2.23	3.04	4.65	5.23
8	0	0	1.81	1.54	4.00	2.52
9	0	ND	2.58	3.52	7.20	4.15
10	0	2.41	0	3.57	4.41	5.63
11	0	1.18	1.40	0	3.79	4.52
12	0	1.54	1.93	0	3.48	4.41
13	PS	1.54	PS	4.68	6.97	8.08
14	PS	4.83	5.34	6.56	7.91	8.04
15	PS	6.80	7.36	6.78	7.77	7.57
16	PS	7.11	7.40	7.28	8.30	8.00
17	PS	4.87	6.11	6.70	7.36	7.52

it is usually impossible or impractical to test all individuals. One has to be content to test a small sample of the population to obtain an estimate of the viral incidence and mean viral level in the population. For examination of fish populations, the sample size is determined by an assumed low (usually 2 or 5%) maximum incidence of the virus, by the size of the population, and by the confidence level (usually 95%) required (Ossiander and Wedemeyer 1973). When the incidence of infection in a population is high and the testing is being done solely to demonstrate the presence or absence of the virus in the population and not to detect the virus in every infected animal, the maturity of the infection in the fish chosen for testing is irrelevant. If the virus is being expressed in detectable levels in some of the fish chosen, it will be isolated and the population determined to contain virus carriers. When the intention of the testing is to describe quantitatively the viral status of the population (percent incidence, mean viral titer, distribution of viral titers), it is essential that the infection be fully mature.

Production and release of IHN virus in adult salmon follows a long period in which the virus cannot be isolated. Virus can be first isolated just as the fish are about to spawn, and for the few days of life remaining after they spawn. Examination of a salmon population spawning in a hatchery means that CF samples are taken from fish in spawning condition. Our results show that the use of such samples for a quantitative estimate of the viral status of the population would probably result in low estimates of the viral incidence and mean titer. Often, however, fish in spawning condition are the only ones available, and they must be used for virological tests. The fish chosen for sampling should then be captured at the end of the spawning migration, when expression of the virus peaks both in individual fish and in the population.

The IHN virus cannot be detected during the ocean phase of the salmon's life cycle, nor during the migration from ocean to freshwater spawning grounds (Mulcahy et al. 1984). The virus is difficult to demonstrate in visceral organs of fish in prespawning condition (Mulcahy et al. 1982), but appears rapidly in CF as ovulation occurs and CF appears. The source of CF and its associated virus is not known. The female sockeye salmon that were sampled daily in this study were initially selected as being in prespawning condition on the basis of the inability to collect CF and eggs. The lack of CF as a criterion for judging spawning condition was effective in synchronizing this experimental group of fish because 90% of the fish selected ripened overnight. Most of the fish on the spawning grounds on the day the fish were captured were probably in the same stage of ripeness.

When individual females were sampled daily for 6 d, there was a clear trend for the proportion of fish with high virus titers to increase (Fig. 2), presumably because the infection was peaking. Athough 41% of the fish had virus levels between 10^8 and 10^9 pfu/mL on the final day, many fish had lower levels, suggesting that adult females vary in their response to infection. Of special interest were the fish that were originally negative for virus or had the lowest levels of virus on the first sampling day. Although there was a general trend for the virus titers of these fish to increase, most of these fish later failed to develop extremely high levels of virus. These fish expressed less virus as a group than those fish that had virus titers of 10^2 pfu/mL or higher on the first day. Perhaps these fish represented the proportion of the population that is genetically resistant to viral infection (McIntyre and Amend 1978). Another possibility is that these fish were initially uninfected, acquired the virus by waterborne transmission (Mulcahy et al. 1983a), and had insufficient time for the infection to develop fully.

The reversion of some fish from positive to negative is of concern for the detection of IHN virus. In fish where the virus was present at low titer, its apparent disappearance can be attributed to the threshold of detection with the virus isolation methods we used. Where the virus was initially present at fairly high levels, other, unknown mechanisms must be responsible.

When a sockeye salmon population was surveyed by sampling only naturally spent fish, the infection rates, mean titers, and titer distributions remained nearly constant over a 6-d period. Using such fish for virus sampling is clearly better than using fish before they spawn. Unfortunately, obtaining naturally spent fish usually requires a special effort that is both costly and time-consuming. Fish being spawned at hatcheries will continue to represent the greater proportion of fish sampled for IHN virus. The interpretation of the virus statistics obtained through testing of other than naturally spent fish should be done with caution and with an appreciation for the kinetics of IHN virus expression in these fish.

In most virus-testing efforts, samples are run as pools of CF from five fish and the presence of the virus is determined by a cell culture isolation method which is not quantitative. With these methods, the only statistic that can be derived is the presence or absence of the virus in the population. Pooling of CF samples prevents the accurate determination of infection rate. When a quantitative test, such as the plaque assay, is used for virus detection, and samples are examined individually rather than as pools, more population statistics can be derived, such as the infection rate, the mean and range of virus titer, and the proportion of samples which have high virus titers (Mulcahy et al. 1983b).

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Prediction of Emergent and Floating-Leaved Macrophyte Cover in Central Swedish Lakes

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From multiple regression analysis of data from Central Swedish lakes, we suggest that the peak areal cover (hectares) of emergent vegetation is predictable from lake surface area, mean depth, and annual mean total nitrogen concentration in the lake water. The data also suggest that the percent cover of emergent macrophytes is predictable from mean depth and total nitrogen. However, the factors influencing floating-leaved macrophyte cover are not as clear.

À partir de l'analyse par régression multiple de données provenant de lacs du centre de la Suède, nous estimons que la couverture superficielle maximale (hectares) de la végétation qui émerge est prévisible si l'on connaît la superficie du lac, la profondeur moyenne et la concentration annuelle moyenne d'azote total dans l'eau du lac. Les données portent également à croire que la couverture en pourcentage des macrophytes qui émergent est prévisible à partir de la profondeur moyenne et de l'azote total. Cependant, les facteurs qui influencent la couverture des macrophytes à feuilles flottantes ne sont pas aussi clairs.

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Macrophytes are a significant component of aquatic primary production (Wetzel 1979; Westlake 1975, 1982) and play important roles in nutrient cycling (Barko and Smart 1980; Gopal 1984) and as a resource for terrestrial and aquatic animals (Sculthorpe 1967; van der Velde and van der Heijden 1985). Furthermore, excessive macrophyte growth is a common water quality problem worldwide (e.g. Chapman et al. 1974; Shireman et al. 1982). However, despite many decades of research (Pearsall 1920; Lohammar 1938; Seddon 1972; Hutchinson 1975; Spence 1982), few quantitative models yet exist which allow us to predict either macrophyte biomass or productivity. As noted by Chambers and Kalff (1985), we are still unable to predict the extent to which macrophyte biomass will change in response to changes in nutrient loading.

It is clear from comparative studies (Lachavanne 1982, 1985) and nutrient enrichment studies (Zdanowski et al. 1975; Granéli 1985) that the biomass and composition of macrophyte communities is dependent in part on the external supply of nutrients. However, macrophyte growth is also strongly influ-

TABLE 1. Summary of data from 23 Central Swedish lakes. Note: these data have been rounded. A copy of the data set used in the regression analyses is available from the authors.

Lake	Area (km^2)	Z (m)	Color (Pt)	COND (µs/cm)	K (mg/m^3)	TN (mg/m^3)	TP (mg/m^3)	CHL (mg/m^3)	Emergent macrophyte cover (ha)	Floating macrophyte cover (ha)
Natsjon	0.023	1.5	46	34	0.6	616	44.4	20.4	—	0.037
Siggeforasjon	0.73	4.4	19	65	0.8	546	12.0	2.8	5.6	1.3
Laksjon	0.025	1.5	74	75	0.8	735	29.2	9.6	0.056	0.11
Vansjon	2.4	—	23	125	1.6	1182	59.4	47.4	71.0	24.0
Mossaren	0.075	1.5	83	150	0.8	1309	29.6	10.8	0.92	2.0
Strandsjon	1.3	1.7	18	166	1.9	1313	120.8	79.0	31.0	29.0
Frosjon	0.28	1.5	57	235	0.8	1123	40.4	15.0	18.0	9.5
Lillsjon	0.038	4.0	9	243	45.4	1000	65.2	13.2	0.31	0.24
Tamnaren	38.0	1.0	20	256	1.9	860	40.0	12.0	595	2358
Skedviken	8.6	3.0	11	254	2.6	1132	61.6	38.8	75.0	15.0
Metsjon	0.45	2.5	18	254	2.0	1024	51.2	33.2	7.4	9.1
Sabysjon	0.36	1.5	16	288	3.4	895	102.4	25.2	9.6	5.4
Valloxen	2.9	4.5	10	302	3.7	1083	34.8	28.8	27.0	6.3
Funbosjon	2.0	3.0	25	392	3.5	1486	85.6	16.4	82.0	58.0
Ekoln	19.0	19.6	18	386	4.5	2225	149.2	31.6	200	58.0
Erken	24.0	9.0	9	278	2.3	609	31.8	6.0	89.0	101
Kundbysjon	0.26	0.4	14	365	4.1	1764	146.6	24.0	17.0	—
Lilla Ullevifjarden	—	—	3	303	4.0	729	35.0	10.4	—	—
Sunnerstaviken	0.10	1.0	13	543	6.6	1804	290.8	80.4	0.74	1.3
Alasjon	0.39	1.0	17	572	5.2	1021	48.4	12.4	23.0	13.0
Karven	1.8	1.5	23	392	4.1	1493	76.0	7.2	70.0	46.0
Brantshammarsjon	0.075	2.0	8	1057	9.4	2028	18.6	5.6	2.8	2.6
Hjalstaviken	1.4	5.0	17	956	10.6	2036	66.0	11.5	111	—

enced by lake morphometry. An increase in lake area usually leads to an increase in the littoral area available for macrophyte colonization, and in a lake of given surface area, the littoral area is a function of the lake mean depth. The purpose of this note is to examine the effects of both nutrients and lake morphometry on the areal cover of floating-leaved and emergent macrophytes in lakes, using data from an extensive survey of macrophyte vegetation in Central Sweden.

Materials and Methods

In 1975–77, 23 lakes in the Uppland area of Sweden were studied (Wallsten 1981) to examine the extent to which their chemistry and macrophyte vegetation had changed since an earlier study by Lohammar (1938). The province of Uppland is part of the Central Swedish Lowlands, and the bedrock is partly covered by wave-washed glacial drift and by glacial and postglacial clay.

Sampling of the lakes for temperature, transparency, and water chemistry was begun in June 1975 and continued at 2- to 4-mo intervals until March 1977. Samples were taken at a depth of 0.25 m in the center of each lake, with the exception of Lake Ekoln. Samples from Lake Ekoln were taken from a shallow bay near the bridge to Skarholmen. Analyses for conductivity, color, and chlorophyll *a* were made on the day of sampling, and further analyses were done later on samples preserved with $HgCl_2$. Analytical methods for the chemical analyses are from Ahlgren and Ahlgren (1976), with the exception of color and potassium (cf. Wallsten 1981).

The distribution and peak areal coverage of emergent and floating-leaved vegetation of 22 of the lakes was mapped in late August 1976, using infrared color photography from the air. A Wild ST 4 stereoscope was used in interpreting the photographs, and 1:10 000 scale maps were used to determine the boundaries of the lake shorelines. The areal cover of the macrophytes was measured twice using photoelectric methods, and peak annual coverage was estimated as the mean of these two measurements. The air photos and vegetation maps are on file at the Institute of Limnology, University of Uppsala.

Annual mean values for water chemistry and chlorophyll *a* were calculated for the period 6 August 1975 – 26 August 1976. These data and the measurements of peak macrophyte areal cover (hectares) for the 22 lakes were transformed to their common logarithms; this transformation was found to be necessary to reduce heteroscedasticity and improve linearity in subsequent regression analyses. Statistical analyses of the data set were made with the SYSTAT statistical software (Wilkinson 1984) on an IBM-PC/AT microcomputer, and an inspection of residual error was used to evaluate the results of regression analyses. Because of the presence of missing values, the number of degrees of freedom in the regression models presented below varied among the analyses. A summary of the data used in the analyses is presented in Table 1.

Results

Effects of lake area on areal cover — Although lake surface area should be an important determinant of macrophyte cover in lakes, no models yet exist in the literature which quantify this relationship. Analyses thus were first made of the effects of lake area on the areal cover of emergent and floating-leaved macrophytes. As can be seen in Fig. 1 and 2, the areal cover of emergent macrophytes (EMERGE, hectares) and of floating-leaved macrophytes (FLOAT, hectares) was strongly correlated with lake area (AREA, square kilometres). Multiple regression analysis indicated that the data could be best fit by the following models (Table 2):

$$(1)\quad \log \mathrm{EMERGE} = 1.508 + 0.991 \log \mathrm{AREA} - 0.317(\log \mathrm{AREA})^2, \qquad r^2 = 0.89$$

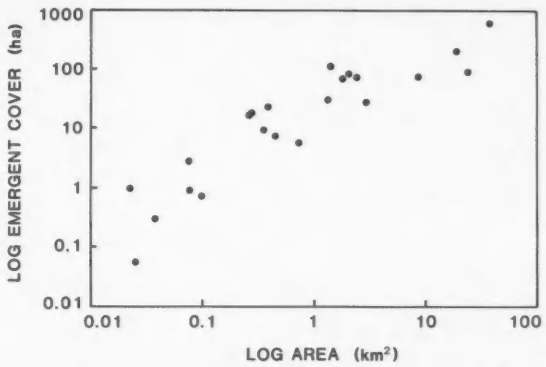

FIG. 1. Relationship between emergent macrophyte areal cover and lake surface area in 22 Central Swedish lakes.

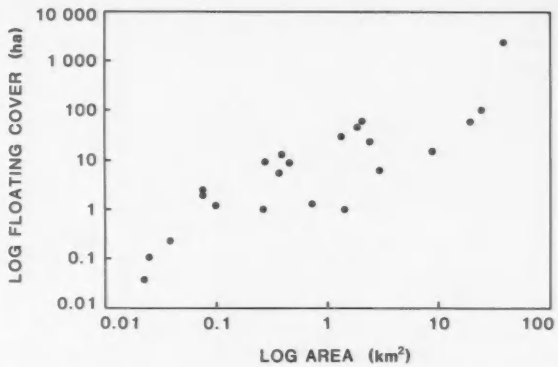

FIG. 2. Relationship between floating-leaved macrophyte areal cover and lake surface area in 22 Central Swedish lakes.

TABLE 2. Results of regression analyses (A) between emergent macrophyte areal cover and lake surface area and (B) between floating-leaved macrophyte areal cover and lake surface area.

A. Dependent variable: log EMERGE (N = 21)

Variable	Coefficient	SE	t	p (two-tailed)
Constant	1.508	0.104	14.54	0.001
Log AREA	0.991	0.085	11.64	0.001
(Log AREA)^2	−0.317	0.089	−3.56	0.002

Analysis of variance

Source	SS	df	MS	F
Log AREA	16.231	1	16.231	135.493
(Log AREA)^2	1.521	1	1.521	12.694
Error	2.156	18	0.120	

B. Dependent variable: log FLOAT (N = 20)

Variable	Coefficient	SE	t	p (two-tailed)
Constant	1.008	0.119	8.44	0.001
Log AREA	0.989	0.122	8.09	0.001

Analysis of variance

Source	SS	df	MS	F
Log AREA	18.170	1	18.170	65.372
Error	5.003	18	0.278	

(2) $\log \text{FLOAT} = 1.008 + 0.989 \log \text{AREA}, \quad r^2 = 0.78.$

Effects of other factors on areal cover — Although macrophyte areal cover was strongly dependent on lake surface area (Fig. 1 and 2), a great deal of residual variance could not be accounted for by Eq. 1 and 2. Stepwise multiple regression analysis was used to determine if this residual variance could in part be accounted for by additional factors.

The additional variables chosen for analysis were annual mean total nitrogen concentration (TN, milligrams per cubic metre), total phosphorus (TP, milligrams per cubic metre), dissolved potassium (K, grams per cubic metre), water color (Pt units), conductivity (COND, microsiemens per centimetre), chlorophyll *a* (CHL, milligrams per cubic metre), and lake mean depth (Z, metres) (Table 1). Nitrogen, phosphorus, and potassium were chosen as the nutrients most likely to limit macrophyte growth (cf. Gerloff 1975), and conductivity was included as an overall index of nutrient availability. Water color and chlorophyll were chosen as factors which potentially reduce light availability for macrophyte germination and growth.

Stepwise regression and residual error analyses suggested that the best models for macrophyte areal cover included lake area, mean depth, and annual mean total nitrogen concentration (Table 3):

(3) $\log \text{EMERGE} = -1.853 + 1.102 \log \text{AREA} - 0.586 \log Z + 1.071 \log \text{TN}, \quad r^2 = 0.91$

(4) $\log \text{FLOAT} = -2.390 + 1.151 \log \text{AREA} - 1.117 \log Z + 1.266 \log \text{TN}, \quad r^2 = 0.91.$

In subsequent analyses, the annual mean concentrations of total phosphorus and dissolved potassium were substituted for total nitrogen to see if these variables could substitute for TN in the model equations. However, in no case was the coefficient for either total phosphorus or dissolved potassium significant ($p > 0.05$).

Models for macrophyte percent cover — The above models indicate that lake surface area, mean depth, and mean annual total nitrogen concentration are the best predictors of the areal cover of emergent and floating-leaved macrophytes. These conclusions are further supported for emergent macrophytes by an analysis of their percent cover (PEMERGE, emergent macrophyte cover/lake surface area), which removes a potential bias in the previous regression analyses caused by the inclusion of lake surface area. The percent cover data were transformed using $\log_{10}$ logit transform (cf. Smith 1986), and a multiple regression analysis was made including mean depth and total nitrogen as candidate variables. As can be seen in Table 4, both variables were significant predictors of emergent macrophyte percent cover. However, as was found in previous analyses of blue-green algae (Smith 1985, 1986), the macrophyte model based on percentage data (Eq. 5) was much less precise than that based on absolute measurements (Eq. 3):

(5) $\text{logit (PEMERGE)} = -6.346 - 0.660 \log Z + 1.926 \log \text{TN}, \quad r^2 = 0.49.$

TABLE 3. Results of multiple regression analyses for macrophyte areal cover.

A. Dependent variable: log EMERGE (N = 20)

Variable	Coefficient	SE	t	p (two-tailed)
Constant	−1.853	1.424	−1.30	0.212
Log AREA	1.102	0.090	12.26	0.001
Log Z	−0.586	0.214	−2.74	0.015
Log TN	1.071	0.457	2.34	0.032

Analysis of variance

Source	SS	df	MS	F
Log AREA	16.751	1	16.751	150.318
Log Z	0.834	1	0.834	7.486
Log TN	0.611	1	0.611	5.483
Error	1.783	16	0.111	

B. Dependent variable: log FLOAT (N = 19)

Variable	Coefficient	SE	t	p (two-tailed)
Constant	−2.390	1.596	−1.50	0.155
Log AREA	1.151	0.101	11.42	0.001
Log Z	−1.117	0.298	−3.75	0.002
Log TN	1.266	0.524	2.42	0.029

Analysis of variance

Source	SS	df	MS	F
Log AREA	18.933	1	18.933	130.396
Log Z	2.037	1	2.037	14.028
Log TN	0.848	1	0.848	5.843
Error	2.178	15	0.145	

As was true in Eq. 3, neither total phosphorus nor dissolved potassium could be substituted for TN in this model or any subsequent multiple regression analysis.

Similar analyses involving floating-leaved macrophytes were not successful. Neither mean depth, total nitrogen, nor any of the other five candidate variables was significantly correlated with floating-leaved macrophyte percent cover.

Discussion

From these analyses, we suggest that three principal factors, lake area, mean depth, and total nitrogen concentration, determine the areal cover of emergent macrophytes in Central Swedish lakes. As discussed above, morphometric effects on macrophyte cover can be expected because of their influence on the area of the littoral zone. However, our data also suggest that nitrogen is the nutrient which most likely limits emergent macrophyte cover in lakes.

Although previous studies have shown a clear dependence of emergent macrophyte biomass on trophic state (e.g. *Phragmites*: Björk 1967; Westlake 1982; *Carex* and *Typha*: Hartland-Rowe and Wright 1975), the identity of the principal growth-limiting nutrient(s) has not been clear. The importance of total nitrogen in Eq. 3 and 5 is consistent with studies suggesting that nitrogen is more likely to be a growth-limiting nutrient for emergent macrophytes than phosphorus (cf. Granéli 1985). It is also important to note that neither the concentration of total phosphorus nor dissolved potassium could be substituted for total nitrogen in Eq. 3 or 5.

TABLE 4. Results of multiple regression analysis for macrophyte percent cover.

Dependent variable: logit (PEMERGE) (N = 21)

Variable	Coefficient	SE	t	p (two-tailed)
Constant	−6.346	1.973	−3.22	0.005
Log TN	1.926	0.638	3.02	0.007
Log Z	−0.660	0.292	−2.26	0.037

Analysis of variance

Source	SS	df	MS	F
Log TN	2.286	1	2.286	9.125
Log Z	1.277	1	1.277	5.096
Error	4.510	18	0.251	

Our conclusions concerning the cover of floating-leaved macrophytes are not as clear. Multiple regression analyses (Eq. 4) indicate that lake mean area, mean depth, and total nitrogen concentration are the best predictors of floating-leaved macrophyte areal cover. However, similar analyses using percent cover were not successful, suggesting that further research is needed to determine the factors influencing the cover of floating-leaved macrophytes in lakes.

Although the data used in this study are from Central Sweden, we anticipate that similar relationships will be evident in other lakes. It is hoped that this study will prompt similar analyses of data from lakes in other geographical regions, so that the effects of other variables such as climate and watershed geology can be explored. We also feel that this approach will be applicable to the analysis of emergent macrophyte cover in other systems such as embayments, estuaries, and streams.

It is important to note that our analyses have considered only the effects of nutrients in the water column. We suggest that future modelling efforts consider sediment nutrients (nitrogen, phosphorus, potassium) as additional candidate variables in order to assess the relative importance of water column and sediment nutrients on emergent macrophyte areal cover. The inclusion of time-series data (e.g. Andersson 1978; Hitchin et al. 1984; Lachavanne 1985) in future models might also allow us to better predict the changes in emergent macrophyte cover which result from changes in trophic state.

Finally, we emphasize that macrophyte areal cover is only a correlate of macrophyte biomass. Evidence from surveys (e.g. Andersson 1978) and nutrient fertilization studies (Solander 1983; Granéli 1985) has shown that macrophyte populations respond to changes in nutrient supply by altering the number of individuals per square metre as well as the dry weight per individual. We anticipate that the approach used here can be used successfully to develop pedictive models for macrophyte biomass as well. This approach may also be useful in developing predictive models for submerged macrophyte biomass and areal cover. However, in the case of submerged macrophytes, we anticipate that light availability will be an important additional factor.

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Selective Predation by *Procladius* in an Arctic Alaskan Lake

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In arctic Alaskan Toolik Lake, the predatory larval chironomid *Procladius* spp. (Diptera) feeds on a variety of benthic organisms including algae, other chironomids, and microcrustaceans. Laboratory experiments with two similar-sized chironomid prey, instar I *Chironomus* and instar II *Paratanytarsus*, showed that *Procladius* was a more effective predator on *Chironomus*. The two prey used different foraging behaviors; *Chironomus* grazed an area around its tube but *Paratanytarsus* remained inside its tube. In a field experiment, in which *Procladius* density was manipulated, a decrease in prey density occurred in the chironomid group Orthocladiinae, which could be attributed to *Procladius* predation. Gut analyses of *Procladius* showed that a higher proportion of Orthocladiinae was eaten than could be predicted from their abundance. Orthocladiinae, which typically graze around tubes, may have been more susceptible to *Procladius* because of behavioral characteristics.

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Dans le lac Toolik, en Alaska, la larve prédatrice du chironomidé *Procladius* spp. (Diptères) se nourrit de divers organismes benthiques dont des algues, d'autres chironomidés et des crustacés microscopiques. Des expériences effectuées en laboratoire avec deux proies de chironomidés de taille semblable, soit Instar I *Chironomus* et Instar II *Paratanytarsus*, ont montré que *Procladius* était un prédateur plus efficace de *Chironomus*. Les deux proies utilisaient des techniques différentes de recherche de nourriture : *Chironomus* broutait un secteur autour de son tube, tandis que *Paratanytarsus* demeurait à l'intérieur de son tube. Dans une expérience sur le terrain, où la densité de *Procladius* a été modifiée, il y a eu diminution de la densité des proies dans le groupe des Orthocladiinés faisant partie des chironomidés, qui pouvait être attribuée à la prédation par *Procladius*. Des analyses de l'intestin chez *Procladius* ont montré que la proportion d'Orthocladiinés qui ont été consommés est plus grande qu'on pouvait le prédire à partir de leur abondance. Les orthocladiinés qui, de façon typique, broutent autour des tubes pourraient avoir été plus susceptibles à la prédation par *Procladius* en raison des caractéristiques de leur comportement.

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The food habits and community role of *Procladius* spp., predatory chironomid larvae in the subfamily Tanypodinae, have been studied by many benthic ecologists. *Procladius* are common in freshwater habitats and are known to feed on a variety of foods, including larval chironomids, oligochaetes, crustaceans, diatoms, desmids, and detritus (Morgan 1949; Hilsenhoff 1967; Kajak et al. 1968; Armitage 1968; Roback 1969; Baker and McLachlan 1979; Dusoge 1980; Kajak 1980; Vodopich and Cowell 1984). Several authors have suggested that *Procladius* may be important in determining the distribution and abundance of its prey (Hilsenhoff 1967; Kajak et al. 1968; Jonasson 1972; Kajak 1980; Dusoge 1980), although Vodopich and Cowell (1984) suggested that physical factors detemine prey distributions. Prey availability to *Procladius* appears to be determined, at least in part, by prey size. In a laboratory experiment, Baker and McLachlan (1979) determined that small (2–5 mm) Chironominae, rather than medium and large Chironominae, were ingested by *Procladius choreus* Meigen. Field studies of *Procladius* indicate that predation is most intense on early instar larvae (Hilsenhoff 1967; Kajak et al. 1968; Jonasson 1972).

I suggest that foraging behavior is another factor affecting susceptibility of chironomids to *Procladius* predation. Foraging behavior has not been thoroughly studied, but the existing evidence suggests that it is diverse among chironomids. Many chironomid species build tubes, and their foraging behaviors are closely linked to these structures. Members of the subfamily Tanypodinae, such as *Procladius*, do not build tubes (Oliver 1971). In the subfamily Chironominae, most species build tubes, and foraging behaviors include grazing areas around the tubes, filtering currents of water drawn through the tubes by undulations of the body, and feeding from convoluted burrows beneath the sediment surface (Walshe 1947; Oliver 1971; Jonasson 1972; McLachlan and Cantrell 1976; Wiley 1978, 1981). Foraging in the subfamily Orthocladiinae is not as well studied, but some graze areas around the outside of their tubes (Wiley and Kohler 1984), and some do not build tubes (Walde and Davies 1984). Chironomids in tubes are less susceptible to stonefly, sculpin, and dragonfly predators than those outside of tubes (Walde and Davies 1984; Hershey and Dodson 1985; Dillon 1985).

My objective was to examine some interactions between instar IV *Procladius* and its chironomid prey in arctic Alaskan Toolik Lake (68°N, 149°W). Both *P.* nr *vesus* Roback and *P. sublettei* var. *grandis* Roback emerged from the study site (Hershey 1985a), but these were not distinguished in the larval stage. In a laboratory experiment, I evaluated the effectiveness of *Procladius* preying on two similar-sized chironomids which differed in their foraging behavior. In a field experiment and with gut analyses, I examined the relationship between chironomid prey eaten by *Procladius* and potential chironomid prey available in the natural habitat.

Methods

In a laboratory experiment, I measured predation rates for instar IV *Procladius* feeding on two different chironomid prey types, instar I *Chironomus* sp. and instar II *Paratanytarsus* sp. These two prey types were used for predation trials because they were similar in size (1 mm in length), could be collected in large numbers, and demonstrated distinct foraging behaviors in the laboratory. Instar I *Chironomus* constructed fixed tubes, but grazed an area outside of their tubes by periodically extending their bodies. *Paratanytarsus* also constructed fixed tubes, but fed by filtering from inside their tubes.

A trial consisted of offering either 10 *Chironomus* or 10 *Paratanytarsus* prey for 24 h to individual instar IV *Procladius* in an 11-cm^2 section of a divided petri dish containing lake water and two drops of a slurry of lake sediment. The prey were introduced into the dishes 24 h prior to predator introduction to allow tube construction. Predation rates were measured as the number of prey eaten per 24 h.

Collecting large numbers of live early instar chironomids from lake sediments is difficult. The small larvae pass through the openings of meshes that will pass sediments, and finding them in unsieved sediments is prohibitively time consuming. Flotation solutions might affect behavior or survivorship. Thus, early instars used as prey were collected from shallow ponds. Instar II *Paratanytarsus* were collected using a sweep net from a small pond near Toolik Lake (Camp Pond). Instar I *Chironomus* hatched from an egg mass collected with a sweep net from a pond near Prudhoe Bay, Alaska. Neither of these species were present in Toolik Lake, although other species of *Paratanytarsus* were present at moderate densities (Hershey 1985a, 1985b). Instar IV *Procladius* were collected from Toolik Lake by sieving lake mud through a 500-μm-mesh net. The sieved material was transferred to a white basin containing lake water; *Procladius* were captured with a pipette. Experimental animals were held for a minimum of 24 h before use.

A predator enrichment experiment was conducted at a 5-m-deep study site in Toolik Lake from 10 to 18 August 1979 to determine if *Procladius* was selective among chironomid prey in the field. Experimental chambers consisted of 10-cm-

diameter × 23-cm-long clear plastic tubes. The tubes were pushed approximately 15 cm into the sediments by a diver and covered with a 2-mm-mesh screen to exclude predatory fish.

There were four experimental treatments. In the high and low *Procladius* treatments, 10 and 5 instar IV *Procladius* larvae, respectively, were added to each of eight tubes. The eight untreated control tubes had no introduction of *Procladius*, and four core samples, the unmanipulated controls, were samples taken at the end of the experiment which were designed to determine whether the tubes affected the natural chironomid community. Prior to enrichment, the instar IV *Procladius* were placed in modified wide-bore syringes filled with lake water. The tapered ends of the syringes had been cut off, and the openings were sealed with stoppers attached to the plungers by strings. A diver introduced the *Procladius* by aiming syringes over the tubes and pushing the plungers.

Tubes were wired together into groups of four to facilitate diver handling, although this technique was abandoned in later experiments at the same site (Hershey 1985a). The four tubes in a group each received the same level of *Procladius* introduction. Groups of tubes were separated on the bottom by a distance of about 2 m; adjacent groups did not receive the same treatment. The experimental design involved pseudoreplication of the type described by Hurlbert (1984) as clumped segregation, but clumps were replicated. Clumped segregation can lead to spurious treatment effects due to differences between clump locations (Hurlbert 1984). Comparison of the two groups of four core tubes which received the same treatment provides some protection against this problem. Examination of *Procladius* gut contents (see below) provides an additional means of determining whether treatment effects could be attributed to *Procladius* predation.

All core samples were retrived by a diver after 8 d by sealing the ends of the tubes with rubber stoppers. Tube contents were washed through a 70-μm-mesh net, a size fine enough to retain all instars of Chironomidae. Samples were preserved with 10% buffered formalin. Sugar flotation, modified from Anderson (1959), was used to aid sample processing. The technique retained approximately 95% of all chironomids. Chironomids were mounted in Amman's lactophenol and identified to genus.

Guts were dissected from all instar IV *Procladius* (196 individuals) retreived from the core samples and slide mounted in lactophenol. All guts were examined for chironomid head capsules which were identified to the lowest possible taxon. A subset of the gut slides (27) was examined for algae and other animal prey, and these food items were also identified to lowest possible taxon. Detritus content of *Procladius* guts and weight and volume of food items were not evaluated.

Predation rates were compared for *Procladius* feeding on *Paratanytarsus* and *Chironomus* using a *t*-test. Chironomids from the field experiment were grouped into the following categories: Orthocladiinae, Chironomini, Tanytarsini, Tanypodinae, and Diamesinae/Prodiamesinae. This was necessary because most genera were absent from many of the samples; thus, generic analyses would have been confounded by the introduction of too many zeros. I compared the number of prey in each category between each group of four core tubes using *t*-tests to check for differences between locations which could not be attributed to *Procladius* treatment. I used one-way ANOVA to compare number of chironomids in each prey category between treatments. For significant ANOVAs, I compared unmanipulated controls, low *Procladius*, and high *Procladius* treatments with untreated controls using Tukey's hsd test. I compared distributions of prey groups found in *Procladius* guts from predator enrichment samples with the expected distribution of prey based on proportion in untreated control samples and unmanipulated controls using a chi-square test.

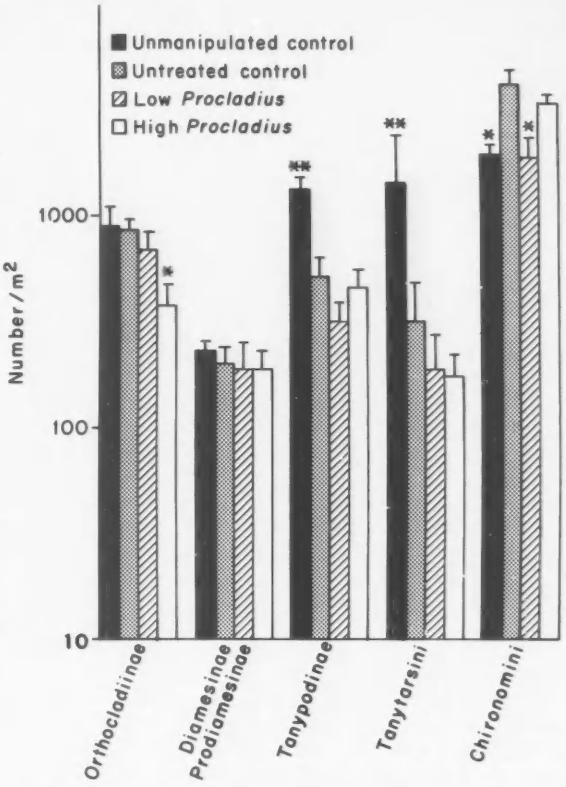

FIG. 1. Densities (mean ± SE) of chironomid prey groups from the predator enrichment experiment. Asterisks show means that are significantly different from the untreated controls using Tukey's hsd test (*$p < 0.05$, **$p < 0.01$). See text for explanation.

Results

In the laboratory, *Procladius* were more effective predators on *Chironomus*, which foraged by grazing outside its tube, than on *Paratanytarsus*, which foraged by filter-feeding from inside its tube. *Procladius* ate 9.2 ± 0.2 ($\bar{X}$ ± SE) *Chironomus*/24 h, but ate only 1.1 ± 0.4 *Paratanytarsus*/24 h. These means were significantly different ($t = 15.2$, df = 10, $p < 0.001$).

Predator introduction effectively elevated *Procladius* densities in low and high enrichments by factors of 4.7 and 9.7, respectively. Mean densities of instar IV *Procladius* were identical (0.8 ± 0.3, $\bar{X}$ ± SE) in unmanipulated controls and experimental controls. Low enrichment samples had 3.6 ± 0.8 instar IV *Procladius* and high enrichment samples had 7.2 ± 0.8 instar IV *Procladius*. However, 5 and 10 instar IV *Procladius* had been added to these two treatments over the backgroud density, suggesting approximately 44 and 36% mortality and/or emigration of introduced animals, respectively.

In the predator enrichment experiment, the two groups of four core tubes which received the same treatment did not differ significantly for prey categories Orthocladiinae, Tanypodinae,

TABLE 1. Prey items from 27 *Procladius* guts which were examined for both animal remains and algal cells. Counts represent the minimum number of organisms needed to account for observed parts.

Taxon	No. of guts with prey type	No. of prey type observed
Algae	21	362
Melosira	15	191
Surirella	16	89
Fragilaria	8	15
Navicula	7	20
Closterium	9	20
Euastrum	1	1
Pediastrum	16	26
Crustaceans	14	18
Copepoda	3	3
Ostracoda	11	15
Chironomidae	14	27

Tanytarsini, and Diamesinae/Prodiamesinae; for these prey categories, the assumption of no differences due to location is strengthened. However, for the prey category Chironomini, densities of prey in the two groups of four core tubes differed significantly for the untreated controls and the low *Procladius* treatment (df = 6, $t = 3.75$ and $t = 3.19$, respectively). Thus, differences in density of Chironomini among treatments cannot be attributed to predation by *Procladius*.

Differences in prey density were detected by ANOVA for the prey categories Orthocladiinae, Tanypodinae, Tanytarsini, and Chironomini (Fig. 1). For Tanypodinae and Tanytarsini, the significant ANOVAs were due to a higher density of prey in the unmanipulated controls than in untreated controls (Fig. 1; Tukey's hsd test, $p < 0.01$ for both groups). Low and high *Procladius* treatments did not differ significantly from untreated controls for Tanypodinae and Tanytarsini (Fig. 1). The differences between untreated controls and unmanipulated controls were due to recruitment of instar I larvae onto the sediments where unmanipulated controls were taken. The presence of the screen over core tubes appears to have hindered recruitment into the experimental tubes. As noted above, interpretation of *Procladius* effects on Chironomini is confounded by local patchiness of these larvae. Density of Orthocladiinae did not differ between the unmanipulated controls and untreated controls (Fig. 1; Tukey's test, $p < 0.05$), probably because they were not recruiting during the expriment. The significant ANOVA was due to a lower number of Orthocladiinae in the high *Procladius* treatment than in untreated controls (Fig. 1; Tukey's test, $p < 0.05$). This result appears to be due to predation by *Procladius*.

Gut analyses showed that *Procladius* eats a variety of algal and animal prey, but among chironomid prey eaten, Orthocladiinae were the most common. Of 27 *Procladius* guts examined for all prey types, 21 guts contained algal cells, especially the diatoms *Melosira* and *Surirella* and the desmid *Pediastrum* (Table 1). Fifteen guts contained crustaceans. Of the 106 guts examined for chironomid prey, 63 contained remains of 80 chironomid prey items (Table 2). Six prey items could not be identified below family, but of those recognizable prey, 57 were Orthocladiinae (Table 2).

Chironomid prey were not eaten by *Procladius* in proportion to their abundance in the environment. Untreated control samples were dominated by Chironomini (Fig. 1) in both groups of four core tubes, but chironomid prey in *Procladius* guts were predominantly Orthocladiinae (Table 2). Comparison of prey in guts from enrichment samples with expected prey based on control samples indicates that the observed ingestion of chironomid prey by *Procladius* was significantly different from that expected based on abundance in the environment ($\chi^2 = 168.8$, $p < 0.005$, Table 3). Ingestion of Orthocladiinae was five times higher than expected and ingestion of Chironomini was six times lower than expected (Table 3).

TABLE 2. Chironomid prey items from 106 *Procladius* guts (43 individuals contained no chironomid prey in guts).

Taxon	No. of guts with prey type	No. of prey type observed
Orthocladiinae	42	57
Heterotrissocladius	15	19
Zalutschia	11	18
Psectrocladius	9	10
Parakiefferiella	7	10
Tanytarsini	7	9
Chironomini	5	5
Stictochironomus	4	4
Phaenopsectra	1	1
Tanypodinae	3	3
Procladius	2	2
Ablabesmyia	1	1
Unidentified parts	6	6
Total	63	80

Discussion

Predation rates of *Procladius* were much higher when feeding on *Chironomus* than when feeding on *Paratanytarsus*. Since these larvae were the same size, the observed difference is most likely due to differences in foraging behavior. The filter-feeding behavior, which does not require the larva to leave its tube, probably protected *Paratanytarsus* from *Procladius* whereas the habit of grazing the area around its tube probably made *Chironomus* very susceptible to *Procladius*. The laboratory predation rates are useful for comparing the relative efficiency of predation on the two prey types, but the observed laboratory rates probably do not correspond to natural predation rates due to the lack of three-dimensional structure in the laboratory. Other laboratory experiments have shown that chironomids in tubes are less susceptible to stonefly, sculpin, and dragonfly predation (Hershey and Dodson 1985; Dillon 1985). The laboratory experiment described here provides further generality to the conclusion that chironomid tube-dwelling behavior protects them from predation. My results also extend previous work to show that differences in behavior among tube dwellers, as well as between tube-dwellers and free-swimming chironomids, affects susceptibility to predation. In the predator enrichment experiment, only for the prey category Orthocladiinae could level of *Procladius* enrichment be related to a decrease in prey density. This result suggests that Orthocladiinae were more susceptible to *Procladius* predation than other chir-

TABLE 3. Chi-square table showing the observed and expected values for chironomid prey groups. Expected values were generated from the proportion of prey types in control samples because proportions in enriched samples may have been influenced by *Procladius* activity. Observed values were from guts of *Procladius* instar IV in enrichment samples. Observed and expected values were compared using a chi-square test for goodness of fit (4 df).

Prey group	Proportion in environment	Observed in guts	Expected in guts	χ^2	p
Tanypodinae	0.16	2	7.8	4.3	
Chironomini	0.54	4	26.5	19.1	
Diamesinae/Prodiamesinae	0.04	0	2.0	2.0	
Tanytarsini	0.11	2	5.4	2.1	
Orthocladiinae	0.16	41	7.8	141.3	
Total	1.01	49	49.5	168.8	<0.005

onomids. Gut analysis gives strong support to this conclusion; 77% of identifiable chironomids in *Procladius* guts were Orthocladiinae (Table 2).

Gut analyses also support the conclusion that differences between treatments for the prey category Chironomini were not *Procladius* effects, since Chironomini rarely were found in *Procladius* guts (Table 2). In the predator enrichment experiment, patchiness of Chironomini confounded interpretation of results. For example, the low *Procladius* treatment had fewer Chironomini than untreated controls, but the high *Procladius* treatment did not differ significantly from untreated controls (Fig. 1). A possible interpretation of this result is that at high density, *Procladius* predators interfered with each other, thereby reducing predator efficiency. This interpretation is unlikely for two reasons: (1) few Chironomini were found in predator guts (Table 2) and (2) if interference occurred, it would have been evident in the analyses of Orthocladiinae as well as Chironomini.

Gut analyses confirm that *Procladius* feeds on a variety of foods. Other workers have consistently reported algal cells in *Procladius* guts (Armitage 1968; Roback 1969; Baker and McLachlan 1979; Dusoge 1980; Vodopich and Cowell 1984). Baker and McLachlan (1979) suggested that *Procladius* ingests algae only when animal prey are unavailable, and Vodopich and Cowell (1984) found that *Procladius* survived for some time on a diet of algae and detritus, but were unable to molt to instar IV on that diet. Other workers have also consistently found microcrustaceans in *Procladius* diets (Morgan 1949; Kajak et al. 1968; Roback 1969; Baker and McLachlan 1979; Dusoge 1980; Kajak 1980; Vodopich and Cowell 1984); thus, feeding on a variety of prey types appears to be a characteristic of *Procladius*. Most other experimental studies of *Procladius* feeding have not considered chironomid prey below the family level, or have studied feeding on a single prey species or on instar I Chironomidae of any species (Kajak et al. 1968; Baker and McLachlan 1979; Dusoge 1980; Vodopich and Cowell 1984). My results extend earlier work to show that chironomid prey groups present in Toolik Lake differ in their susceptibility to *Procladius* predation and that foraging behavior is important in determining susceptibility, even among tube dwellers. Further studies of chironomid foraging behavior are needed to improve understanding of chironomid predator–prey interactions.

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Survival from Sea Lamprey (*Petromyzon marinus*) Predation by Two Strains of Lake Trout (*Salvelinus namaycush*)

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Swink, W. D., and L. H. Hanson. 1986. Survival from sea lamprey (*Petromyzon marinus*) predation by two strains of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 43: 2528–2531.

We tested the hypothesis that the better survival of the Seneca Lake strain of lake trout (*Salvelinus namaycush*) in Lake Ontario resulted from their greater resistance than other stocked strains to sea lamprey (*Petromyzon marinus*) predation. No significant difference was found in the survival of Marquette and Seneca Lake strains of lake trout subjected to single sea lamprey attacks in the laboratory. Of 85 Marquette strain lake trout, 44% died as a direct result of sea lamprey predation, 20% died of undetermined causes, and 36% survived; for 90 lake trout of the Seneca Lake strain, the respective percentages were 39, 21, and 40. The location of sea lamprey attachment on the lake trout was not a factor in mortality, which was significantly higher at water temperatures of 15.6–17.8°C than at 0.6–15.6°C. Our study suggested that the seemingly greater survival rate of Seneca Lake fish in Lake Ontario probably resulted from differences in behavior or environmental preferences that decreased their exposure to sea lamprey attacks.

Les auteurs ont testé l'hypothèse voulant que le meilleur taux de survie de la lignée de touladi (*Salvelinus namaycush*) du lac Seneca dans la lac Ontario résultait d'une plus grande résistance, comparativement aux autres lignées introduites, à la prédation par la lamproie marine (*Petromyzon marinus*). Aucune différence significative n'a été notée entre les taux de survie des lignées de touladi Marquette et lac Seneca soumis en laboratoire à une seule attaque par des lamproies marines. Des 85 touladis de lignée Marquette, 44 % sont morts des effets directs de la prédation par les lamproies, 20 % de causes indéterminées et 36 % ont survécu; quant aux 90 touladis de lignée lac Seneca, ces pourcentages étaient respectivement de 39, 21 et 40. Le point d'attache des lamproies n'avait pas d'effet particulier sur la mortalité. La mortalité des touladis était significativement supérieure aux températures de l'eau se situant entre 15,6 et 17,8°C comparativement aux températures de 0,6 à 15,6°C. La présente étude porte à croire que la plus importante survie de la lignée lac Seneca dans le lac Ontario s'explique par des différences de comportement ou des préférences environnementales qui ont pour effet de réduire l'exposition des poissons aux attaques des lamproies.

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The collapse of the lake trout (*Salvelinus namaycush*) population in Lake Ontario in the 1940's probably resulted from commercial overharvest. Sea lamprey (*Petromyzon marinus*) predation apparently affected the population only after the number and average size of lake trout were reduced by overfishing (Christie 1973).

Sea lamprey predation was considered a major factor in the failure of attempts to reintroduce lake trout into Lake Ontario in the 1950's and 1960's (Schneider et al. 1983). As a result of these failures, the Great Lakes Fishery Commission extended its sea lamprey control program to Lake Ontario in 1971. Although the control measures reduced the sea lamprey wounding rate on lake trout in Lake Ontario, the rate continued to be higher than in Lakes Michigan and Superior.

Stocking of yearling lake trout in Lake Ontario was resumed in 1973. Since that time, six strains of lake trout (Clearwater Lake, Green Lake, Lake Manitou, Lake Michigan, Marquette, and Seneca Lake) have been released to provide genetic diversity and to compare the performance of the different strains (Schneider et al. 1983). For 1973–83, the annual survival of the 1972 and 1975 year classes averaged 60–80% for the Seneca Lake strain but only 30–40% for the other strains. In addition, assessment netting showed that Seneca strain fish of age 9 and older remained abundant whereas few fish of the other strains were older than age 6 (Schneider 1985). The survival rate of the Seneca strain fish was high despite the continued presence of large numbers of sea lampreys. This suggested to some workers that the Seneca Lake strain of lake trout was less vulnerable or more resistant to sea lamprey predation (Schneider et al. 1983). To test this hypothesis, we conducted a laboratory study from 24 April to 21 December 1984 to compare the survival, after a single sea lamprey attack, of lake trout of the Seneca Lake and Marquette strains.

Materials and Methods

The studies were conducted in twelve 151-L aerated aquaria with flowing water from Lake Huron. Water temperature was continuously recorded on a thermograph and fluctuated with the ambient lake water temperature. Mean daily water temperature increased from 0.6 to 13.9°C between April and June,

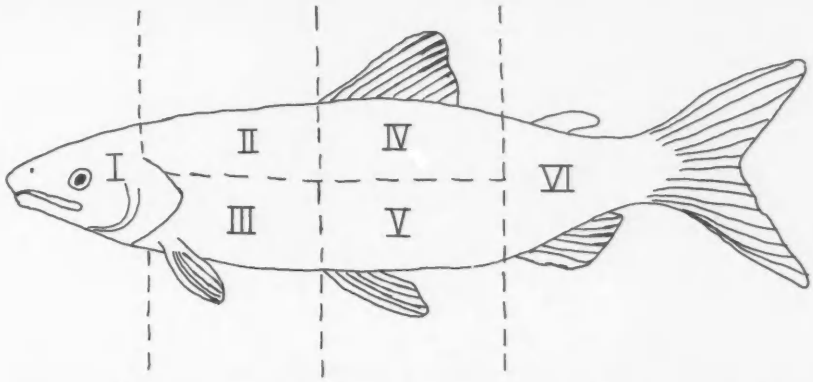

FIG. 1. Body regions of the lake trout used to classify the location of sea lamprey attachment.

fluctuated between 13.9 and 17.8°C during July and August, and declined rather steadily from 14.4 to 2.8°C from September to December.

Sea lampreys used early in the study were recently metamorphosed specimens taken from Great Lakes tributaries. Additional parasitic-stage sea lampreys were obtained from a local commercial fisherman. The Seneca Lake and Marquette strains of lake trout, which were of the 1981 year class, were obtained from the Jordan River National Fish Hatchery, Elmira, Michigan.

Sea lampreys were weighed to the nearest 0.1 g. Lake trout were anesthetized with a 50 $mg \cdot L^{-1}$ solution of MS-222 in Lake Huron water and weighed to the nearest gram. One sea lamprey and one lake trout were placed into each of the 12 aquaria. Observations to check for sea lamprey attachments and detachments were made at least three times a day between 08:00 and 16:30 on weekdays and once a day on weekends. When an attachment occurred, the time and location of attachment (Fig. 1) were recorded within the nearest half hour of observation. Sea lampreys were allowed to make only one attachment on each lake trout.

When a sea lamprey detached, it was reweighed and returned to the aquarium (it was replaced only if it died); the lake trout was removed from the aquarium and replaced with a fish of the opposite genetic strain. We alternated strains of lake trout to avoid bias that might occur because of the large variation in the growth of the sea lampreys.

Lake trout that died during an attachment were weighed and measured and classified according to King (1980). We estimated the wound size on fish that survived an attack and held the fish for 14 d in an 813-L tank filled with Lake Huron water that was maintained at ≤16.1°C. Fish that survived for 14 d were anesthetized and weighed. Throughout the study, lake trout were fed a daily ration of about 1% body weight of dry Silver Cup[1] trout pellets (Murray Elevator, Murray, Utah).

Records on lake trout attacked during the study were placed in three categories: survival, lamprey-induced mortality, and undetermined mortality. Lake trout that lived through the 14-d recovery period were listed in the survival category. A classification as lamprey-induced mortality depended on whether the wound penetrated the skin (Type A wound) or was only an abrasion (Type B wound). Lamprey attacks that resulted in Type A wounds were considered to cause lake trout mortality if (1) there was no fungus present and (2) the lake trout either died while the sea lamprey was attached or died in the recovery tank without being subjected to any other stress (e.g. high water temperature). Type B wounds were considered fatal only if the lamprey was attached for more than 100 h, gained considerable weight, and was still attached when the lake trout died. The undetermined mortality group included the lake trout whose deaths could not be attributed solely to sea lamprey attacks because of secondary fungal infections or failure of the refrigeration unit in a recovery tank.

The chi-square test (Conover 1971) was used to determine the significance of differences in the genetic strain of lake trout, location of lamprey attachment, or water temperature as a factor in lake trout mortality. Lake trout weight, sea lamprey weight, sea lamprey growth, length of time of sea lamprey attachment, and ratio of host weight to sea lamprey weight were segregated by genetic strain of lake trout and the means were compared by the Mann–Whitney *U* test (Conover 1971). Correlations among sea lamprey attachment time, lake trout weight, and sea lamprey growth were made by Spearman's rank correlation coefficient (Conover 1971).

Results

Lake trout mortality — There was no significant difference between the mortalities of Marquette and Seneca Lake strains of lake trout subjected to sea lamprey predation ($\chi^2 = 0.325$, 2 df, $P > 0.50$). Of 85 Marquette strain lake trout attacked by sea lampreys, 44% died as a result of sea lamprey predation, 20% died of undetermined causes, and 36% survived. Of 90 Seneca Lake lake trout attacked by sea lamprey, 39% were killed, 21% died of undetermined causes, and 40% survived. Failure of a refrigeration unit in a recovery tank may have contributed to the delayed mortality of three fish of the Seneca Lake strain.

Since there was no significant difference in mortality between the two genetic strains, mortality data were pooled and analyzed according to the location of the wound (Table 1). Wound location had no significant effect on lake trout mortality ($\chi^2 = 4.165$, 10 df, $P > 0.90$).

Mean daily water temperatures were between 15.6 and 17.8°C for 52 d between 2 July and 28 August. Of 53 lake trout attacked by sea lampreys during this period, 53% died from sea

[1]Reference to trade names does not imply U.S. Government endorsement of commercial products.

TABLE 1. Location of single sea lamprey wounds on body of lake trout, and effect on lake trout survival.

Wounds			Fate of host lake trout (%)		
			Mortality (and cause)		
Body region[a]	No.	Percent	Lamprey	Undetermined	Survival
I	23	13	39	13	48
II	45	26	51	16	33
III	35	20	43	23	34
IV	18	10	39	22	39
V	22	13	36	23	41
VI	32	18	34	25	41
Total	175		42	20	38

[a]See Fig. 1 for location of regions.

lamprey predation and 38% from undetermined causes; 9% survived. The mortality rate during this period differed significantly ($\chi^2 = 29.970$, 2 df, $P < 0.005$) from that when temperatures were below 15.6°C. Of 122 lake trout attacked when mean daily water temperatures were below 15.6°C, 36% died from lamprey attacks and 13% from undetermined causes; 51% survived. Mortality of lake trout in the temperature range of 15.6°C–17.8°C was greater even though the mean weight of the sea lampreys at attachment (37.7 g) was smaller than that of lampreys used at lower temperatures (72.2 g).

The predator–host weight ratio had little influence on lake trout mortality rates. The mortality rate when the predator–host weight ratio was ≥50 was not significantly different from the mortality rate when the ratio was <50 for either the Marquette strain ($\chi^2 = 1.078$, 2 df, $P > 0.25$) or the Seneca Lake strain ($\chi^2 = 0.122$, 2 df, $P > 0.25$). Use of a predator–host weight ratio of 10 as the dividing point also resulted in no significant difference in mortality for the Marquette ($\chi^2 = 3.496$, 2 df, $P > 0.10$) or the Seneca Lake ($\chi^2 = 0.127$, 2 df, $P > 0.25$) strain.

Sea lamprey predation and growth — Sea lamprey attacks on Seneca Lake strain lake trout lasted significantly longer ($m = 85$, $n = 90$, $P < 0.01$) and resulted in significantly greater weight gains by sea lamprey ($m = 84$, $n = 90$, $P < 0.01$) than attacks on Marquette strain lake trout (Table 2). There was no significant difference ($m = 85$, $n = 90$, $P > 0.30$) in the initial weight of the sea lampreys feeding on the two strains of lake trout (Table 2). This was expected, since each sea lamprey was allowed to feed alternately on the two strains. Comparison of the initial weight of the two strains of lake trout (Table 2) showed that the Seneca Lake strain fish were significantly larger ($m = 85$, $n = 90$, $P < 0.01$) than the Marquette strain fish. Consequently, the ratio of host weight to sea lamprey weight (Table 2) was significantly greater ($m = 85$, $n = 90$, $P < 0.05$) for the Seneca Lake strain fish than for the Marquette strain fish.

Sea lamprey growth was highly correlated with attachment time ($r_s = 0.49561$, $P < 0.01$). In addition, initial lake trout weight, for both strains combined, showed a significant correlation with attachment time ($r_s = 0.18477$, $P < 0.05$) and with growth of the sea lamprey ($r_s = 0.25351$, $P < 0.01$).

Lake trout predation on sea lamprey — Lake trout ate 33 sea lampreys during the study: 26 before they made an attachment and 5 after they detached. All were recently transformed lampreys less than 186 mm long. Lake trout were never observed to attack larger lampreys.

Discussion

Sea lamprey predation resulted in considerable mortality of both genetic strains of lake trout in this study. Of the 175 lake trout, 62% died either directly from sea lamprey attacks or as a result of secondary infections. Farmer et al. (1975) reported similar results for a laboratory study: 56% of 35 lake trout died from sea lamprey predation.

The similar mortality rates recorded for the two genetic strains of lake trout indicated that there was no significant difference in their ability to survive single sea lamprey attachments. The similar mortality rates of the two strains occurred despite the Seneca Lake strain's significantly greater ratio of host weight to lamprey weight, which should have further enhanced the Seneca Lake strain's ability to survive. Consequently, the apparently higher survival rate of Seneca Lake strain fish in Lake Ontario was probably not caused by a greater resistance to lamprey attacks. Differences in behavior or environmental preferences between the strains may keep the Seneca Lake strain fish separated from large concentrations of lampreys. This hypothesis is supported by sea lamprey wounding data collected in Lake Ontario during 1978–84. Among fish of the two length groups (532–632 and 633–733 mm), those of the Seneca Lake strain had significantly fewer sea lamprey wounds than did those of the other lake trout strains (C. P. Schneider, New York Department of Environmental Conservation, Cape Vincent, NY, pers. comm.).

The distribution of sea lamprey wounds on lake trout in our study differed from that in previous laboratory studies. Wound location was fairly uniform; excluding head and caudal attachments, approximately equal numbers of wounds were above and below the lateral line. Studies conducted in aquaria by Lennon (1954) on 512 rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) showed that almost 60% of the sea lamprey attachments occurred above the lateral line. In contrast, Farmer and Beamish (1973) observed that 69% of sea lamprey attacks on various teleosts occurred on the ventral surface. Sites of lamprey attachments recorded in the wild have been fairly constant: wounds on the ventral surface (below the lateral line) accounted for 75% of the attacks on rainbow trout taken from Lake Huron (Lennon 1954), 90% of the 180 wounds observed on Lake Huron rainbow trout (Berst and Wainio 1967), and 83% of the sea lamprey wounds on Atlantic salmon (*Salmo salar*) from Love Lake, Maine (Davis 1967). Lennon (1954) suggested that dorsal attacks were more often lethal and suggested that this greater lethality accounted for the preponderance of ventral scars on surviving fish. Laboratory studies of single sea lamprey attacks on lake trout by Parker and Lennon (1956) showed that most lethal attacks occurred on the dorsal surface. However, our study suggested that wound location did not affect the ability of lake trout to survive a sea lamprey attack.

Host survival was affected by water temperature. The percentage of fatal sea lamprey attacks was significantly greater when the temperature was above 15.6°C than when it was lower. Farmer et al. (1977) also found that the rate of host mortality increased with temperature and attributed this increase to an increased blood consumption by feeding sea lampreys.

Sea lampreys used in this study fed longer and had greater growth per attachment while feeding on Seneca Lake strain

TABLE 2. Mean sea lamprey attachment time, growth, and initial weight, initial lake trout weight, and ratio of host weight to sea lamprey weight (ranges in parentheses) observed in aquarium studies of single sea lamprey attacks on two strains of lake trout between 24 April and 21 December 1984.

Lake trout strain and number of attacks	Sea Lamprey			Lake trout weight (g)	Ratio of host weight to lamprey weight
	Attachment time (h)	Weight increase (g)	Initial weight (g)		
Marquette, 85	138.7 (7.0–1632.0)	5.6 (−7.5–40.2)	63.6 (2.2–169.5)	630.7 (217.0–1020.0)	24.9 (3.4–205.5)
Seneca Lake, 90	189.9 (6.0–2229.5)	10.0 (−5.1–55.8)	59.8 (2.0–168.3)	926.5 (344.0–1621.0)	40.1 (5.3–339.0)

than on Marquette strain lake trout. However, greater initial host size, rather than genetic adaptations, probably allowed the lampreys to feed longer on Seneca Lake fish.

Lake trout sometimes ate small sea lampreys, but not larger ones. Not all attempts to eat lampreys were successful; sea lampreys were occasionally observed escaping between the gill arches and operculum of the fish. Farmer and Beamish (1973) reported that sea lampreys avoided ingestion by splake (*Salvelinus namaycush* × *S. fontinalis*), burbot (*Lota lota*), and walleye (*Stizostedion vitreum vitreum*) by escaping through this route. Since sea lampreys longer than 185 mm were not attacked and smaller individuals occasionally escaped, lake trout are probably not a major predator on sea lampreys in the wild.

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Prediction of Angler Success in an Atlantic Salmon, *Salmo salar*, Fishery Two Fishing Seasons in Advance

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The number of young Atlantic salmon, *Salmo salar*, in the spring in two lakes at the headwaters of the Gander River, Newfoundland, was positively correlated ($r = 0.99$) with the number of smolts in the subsequent seaward migration over a 5-yr period. Angler success (catch per unit effort) in the grilse fishery on the Gander River over a 7-yr period was positively correlated ($r = 0.81$) with the number of young in the lakes prior to the opening of the angling season of the previous year. Measures of the abundance of young salmon in freshwaters prior to the smolt run can be used to predict the size of the smolt run and allow for compensatory modifications to the adult fishery a year in advance of acticipated low adult returns.

On a établi une corrélation positive ($r = 0,99$) entre le nombre de jeunes saumons de l'Atlantique (*Salmo salar*) au printemps dans deux lacs à la source de la rivière Gander (Terre-Neuve) et le nombre de saumoneaux présents lors de la migration ultérieure vers la mer au cours d'une période de cinq ans. On a trouvé une corrélation positive

(r = 0,81) entre le succès des pêcheurs à la ligne (prises par unité d'effort) lors de la pêche des madeleineaux sur la rivière Gander pendant une période de sept ans et le nombre de jeunes présents dans les lacs avant l'ouverture de la saison de pêche à la ligne de l'année précédente. On peut se servir des mesures de l'abondance des jeunes saumons présents dans les eaux douces avant la migration des saumoneaux pour prédire le nombre de saumoneaux qui migrent et pour permettre que des modifications compensatoires soient apportées à la pêche des adultes une année avant les faibles remontes prévues d'adultes.

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The number of Atlantic salmon, *Salmo salar*, returning to some rivers to spawn as grilse after a year in the ocean has been shown to be directly related to the number caught in the recreational fishery (Chadwick 1982) and to the number that had left freshwater as smolts in the previous year (Chadwick et al. 1978; Ryan 1986). Angler success, as defined by catch per unit of effort, can be considered as an index of stock abundance (Ricker 1975; Ryan 1986). The ability to predict angler success allows managers time to plan modified seasons or bag limits so that sufficient spawning escapement occurs and assists anglers in their choice of future fishing locations.

Previously, forecasters could predict adult returns only one fishing season in advance (Chadwick 1985). In this report, it is shown that the number of young salmon in freshwater prior to the smolt run can be used to predict the number of fish in the seaward migration and, two fishing seasons in advance, the resultant angler success in the grilse fishery. This technique can provide, for practical purposes, an effective doubling of the time available for compensatory modification to a fishery when low adult returns to a river are expected.

Methods

In the present study, data pertaining to salmon prior to their entry to the ocean are those described in detail by Ryan (1984, 1986) with the inclusion of some data subsequently obtained using identical methods. In summary, the freshwater forms of Atlantic salmon in two central Newfoundland lakes were censused for density and age composition in the spring and again in the fall by Schnabel's multiple, mark–recapture method (Ricker 1975). Spring censuses were completed, usually by the end of May, in advance of the opening of the recreational salmon fishery. The physical and chemical limnology of the two lakes, at the headwaters of the Gander River system, has been described by Ryan and Wakeham (1984). Salmon in Headwater Pond (48°16′N, 55°29′W, 76.1 ha, mean depth = 1.1 m) were censused from 1979 to 1985, and those in Spruce Pond, 3.5 km downstream (48°19′N, 55°28′W, 36.5 ha, mean depth = 1.0 m), were censused from 1978 to 1985. Data from both lakes were combined to compensate for the interlake movements which occur (Ryan 1986). Each year's smolt migration from the two lakes over the period 1979–83 was calculated as the net decrease in number of fish over the spring to fall period, stratified by age group, and shown to be indicative of the abundance of adults returning to the Gander River the following year (Ryan 1986).

Data on the Gander River grilse fishery represent the observed or known fishing effort and catch throughout the entire river from 1979 to 1982 as described by Ash and Tucker (1984) with the inclusion of unpublished data subsequently obtained by identical methods and provided by M. F. O'Connell and E. G. M. Ash (Department of Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld. A1C 5X1).

In the present study, it was hypothesized that the number of fish present in the lakes in the spring was a correlate of the size of the subsequent smolt run and thus would serve as a predictor of angler success two fishing seasons in advance. This was tested by comparing the calculated number of smolts with the number of salmon in the lakes prior to the smolt run in the spring of the corresponding year. This comparison was made using the data analyzed in Ryan (1986) for the period 1979–83. Subsequently, spring salmon abundance and observed angler success (number of grilse captured per rod per week) in the following year were compared, with the inclusion of an additional year's data on spring abundance and angler success. Least-squares regressions (Dixon and Massey 1969) were employed for estimation and comparisons.

Results

The calculated number of smolts which had migrated from the lakes over the spring to fall period was a significant ($P < 0.01$) positive correlate of the abundance of salmon in the lakes in the spring for the period 1979–83 (Fig. 1). Ninety-seven percent of the variation in the number of emigrants was accounted for by the number of salmon present in the lakes prior to the migration. Thus, a measurement of standing stock prior to the smolt run could be used to predict the magnitude of the smolt run.

An estimate of salmon abundance in Headwater Pond in the spring of 1978 was made to increase the sample size for the examination of the relationship between angler success in year $N + 1$ and the number of salmon in both lakes in the spring of year N. The number of salmon in Headwater Pond (Y) was regressed on the number in Spruce Pond (X) using the data from the 14 concurrent censuses from 1979 to 1985. The resulting statistically significant ($P < 0.05$) equation, $Y = 50.007 + 0.5712X$, $r = 0.624$, coupled with the 1978 spring census in Spruce Pond, indicated a population size of 4561 salmon in both lakes in the spring of 1978. This figure was used to complete the time series of spring salmon abundance from 1978 to 1984.

Angler success in the Gander River grilse fishery from 1979 to 1985 was a significant ($P < 0.05$) positive correlate of the number of salmon present in the study lakes in the spring of the preceding year (Fig. 2). More than 66% of the variation in angler success was attributable to salmon abundance in the lakes prior to the opening of the angling season in the previous year. Thus, angler success in a given year could be predicted two fishing seasons in advance.

Discussion

Previously, predictive relationships available to forecast the

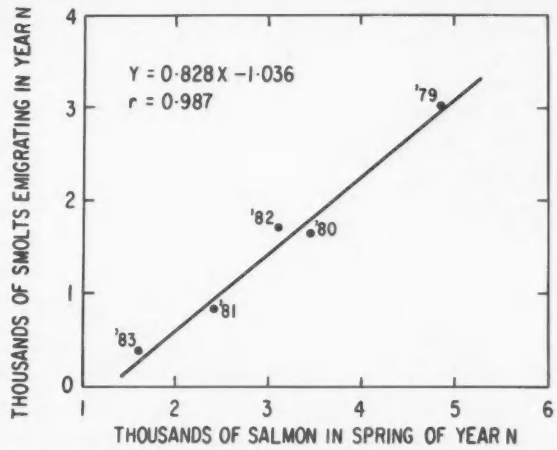

FIG. 1. Calculated number of smolts from Headwater and Spruce ponds related to the number of salmon present prior to the migration. Years of estimates are indicated.

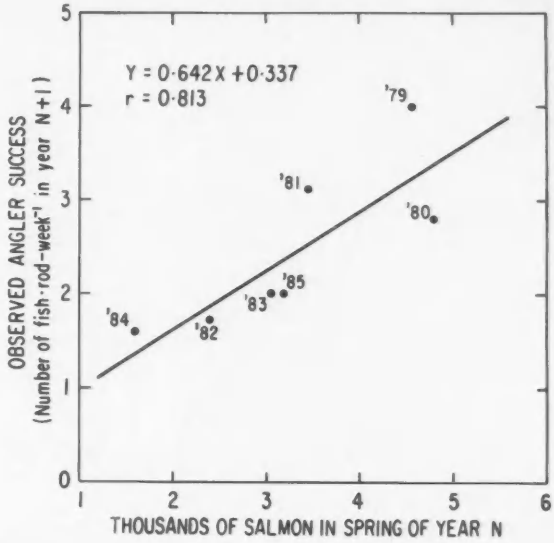

FIG. 2. Observed angler success in the Gander River grilse fishery related to the abundance of salmon in the study lakes prior to the opening of the angling season in the preceding year. Years of angling are indicated.

abundance of sea-run Atlantic salmon returning to spawn as grilse were based upon the number of smolts moving seaward through counting facilities in the previous year (Chadwick 1985) or, in the absence of such facilities, the differences in population structures of young salmon before and after the smolt run of the previous year (Ryan 1986). These relationships could provide estimates only one fishing season in advance, since the opening of commercial oceanic and recreational river fisheries (Pippy 1982; Anonymous 1985) precedes the completion of the smolt run (Chadwick 1981; O'Connell et al. 1983).

Knowledge of stock abundance prior to the smolt run can provide, for practical management purposes, an effective doubling of the time available for compensatory modifications to a fishery when low adult abundance is expected. For example, the capability to forecast angler success two seasons in advance would provide fisheries managers with time to reduce the season or bag limit in the fishery of the year prior to the year of expected low adult abundance. The resulting increased river escapement could result in an increased abundance of young, particularly if the stock was below the carrying capacity of the freshwater environment (Chadwick 1985). These young would partially compensate for the low numbers produced by the adults returning in the year of low adult abundance. In addition, harvests could be further reduced in the year of low abundance. Similarly, a two-season predictive capability would assist anglers in their choice of fishing locations for the following year.

It is apparent from the present study that a spring census of young salmon prior to the smolt run can provide an estimate of the subsequent abundance of smolts. Thus, a spring census may provide a substitute for smolt counts on river systems where the installation of counting fences is not practical due to prohibitive costs or frequent flooding.

It is possible that stock abundance prior to the smolt run could be monitored without conducting the mark–recapture experiments used in the present study. For example, fyke-net catches are well correlated with the population densities of salmon in Headwater and Spruce ponds (Ryan 1984) and thus might serve as indices of the abundance of salmon prior to the smolt run in lakes of other river systems. Appropriate data could be obtained in conjunction with others routinely collected over the long term on index rivers used for monitoring salmon stocks (Chadwick 1985). Such data would be of particular benefit on those river systems characterized by large amounts of standing waters, such as those of Newfoundland.

Acknowledgments

A large number of individuals, especially L. J. Cole, D. P. Riche, and D. Wakeham, assisted with salmon population estimates and data analyses. M. F. O'Connell and E. G. M. Ash continued their provision of unpublished data from the Gander River fishery. H. Mullett drafted the figures. J. B. Dempson, T. R. Porter, D. G. Reddin, and three anonymous individuals constructively reviewed early drafts of the manuscript.

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Some Remarks on "Model of Monthly Marine Growth and Natural Mortality for Babine Lake Sockeye Salmon (*Oncorhynchus nerka*)" by Furnell and Brett

Furnell and Brett (1986) calculated monthly natural mortality rates for the marine life history stages of Babine Lake sockeye salmon (*Oncorhynchus nerka*) by assuming that the exponential rate of mortality, M, is inversely proportional to wet body weight, W, i.e. $M \sim W^{-1.0}$. These rates were used by Brett (1986) to calculate the production energetics of sockeye salmon. The assumption that mortality is a decreasing function of size is certainly correct; whales must have lower mortality rates than bacteria, otherwise they would rapidly become extinct. However, the assumption that the weight exponent is -1.0 is incorrect. There is a substantial body of published evidence that supports an exponent far closer to -0.25 than -1.0.

Ursin (1967) was the first to measure the empirical relationship between mortality rate and body weight in fish; he observed that for adult fishes with an asymptotic wet weight, W_∞, of the order of 10^3 g, $M \sim W_\infty^{-0.33}$. Fenchel (1974) observed that the intrinsic rate of natural increase, r_m, of 43 species of unicellular, heterotherm, and homoiotherm organisms decreased with wet weight as $r_m \sim W^{-0.275}$. Blueweiss et al. (1978) reported a weight exponent of -0.26 for a larger set of observations. In a steady-state system the rate of natural increase must be balanced by an equal force of mortality, which implies that natural mortality rates should decrease with weight to the power of -0.26–0.275. Banse and Mosher (1980) reported that the annual production/biomass ratios (P/B) of 48 species of aquatic invertebrates decreased with wet body weight at maturity, W_S (measured in kilocalories), as $P/B \sim W_S^{-0.37}$. The weight exponent for 11 species of fishes was -0.26. The P/B ratio is equivalent to mortality because in a steady-state system it is that fraction of the annual production that is excess to the needs of maintaining a stable population biomass and which is lost to predation. Peterson and Wroblewski (1984) derived an expression for the weight dependence of mortality in the pelagic marine ecosystem from biomass spectrum theory (Platt 1985). The equation was M (yr^{-1}) = 1.92 (yr^{-1}) $W^{-0.25}$, where W is dry weight (grams). The relationship describes the trend of natural mortality with weight surprisingly well. I have plotted M (d^{-1}) against dry weight (grams) for 199 populations of pelagic marine fish eggs and larvae, juvenile, and adult marine fishes, whales, and marine pelagic invertebrates (McGurk 1986, fig. 2.1). It is clear from Fig. 1 that a linear regression of $\log_e$ (mortality) on $\log_e$ (dry weight) would have a slope indistinguishable from -0.25. In summary, the empirical studies described above give a range of weight exponents of -0.25–0.33, with -0.25 being the most likely value. There is no evidence to support an exponent of -1.0.

If Peterson and Wroblewski's model is the best description of the overall trend of mortality in the sea, then Furnell and Brett's mortality estimates for the 14 mo of the 3_2 life history type, and the first 14 mo of the 4_2 type, should be higher

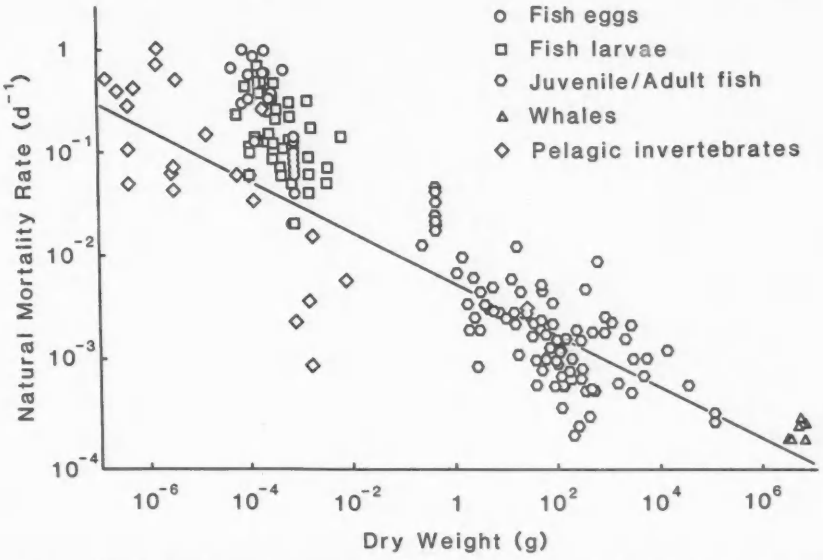

FIG. 1. Plot of instantaneous daily natural mortality rates on dry weight for marine organisms taken from McGurk (1986, fig. 2.1). The line is the predicted mortality rate from Peterson and Wroblewski (1984): M (d^{-1}) = 5.26 (d^{-1}) × $10^{-3} W^{-0.25}$ (g).

than the actual values, and the estimates of the later months should be lower than the actual values. This is indeed the case. The average mortality rate calculated from Peterson and Wroblewski's model (setting dry weight equal to 20% of wet weight) for the 3_2 life history type and the first 14 mo of the 4_2 life history type was $0.076 \cdot mo^{-1}$, about one third of Furnell and Brett's estimate of $0.198-0.214 \cdot mo^{-1}$. The average rate for the final 12 mo of the 5_2 life history type was $0.035 \cdot mo^{-1}$, 8.8 times higher than Furnell and Brett's estimate of $0.004 \cdot mo^{-1}$. Furnell and Brett admit that their natural mortality rates were generally higher during early marine life and lower during later months than the estimates from other methods summarized by Ricker (1976). This suggests that Furnell and Brett's estimates are in error. The root cause of the error was the assumption of a simple inverse relationship between mortality and body weight — Michael D. McGurk, *Institute of Animal Resource Ecology, University of British Columbia, 2204 Main Mall, Vancouver, B.C. V6T 1W5.* (J8799a)

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Reply

In responding to Mr. McGurk's comment on our analysis of the natural mortality experienced by Babine Lake sockeye salmon (*Oncorhynchus nerka*), we would accept his information as generally valuable in examining the relationship between mortality and size in fishes. As emphasized in our presentation, we do not believe a simple inverse relationship (i.e. $M \sim 1/W$) is entirely satisfactory. To quote: "The evidence . . . suggests that a negative size mortality relationship exists. The existence of such a relationship with unknown form should be an impetus for future research. To assume an inverse relationship is perhaps simplistic and ignores the hidden assumptions of . . ." and "Establishing the precise form of this relationship is clearly an important focus for improving understanding of the natural mortality experienced by sockeye salmon."

We do not, however, share Mr. McGurk's confidence in the value of −0.25 for the exponent of this relationship. Mr. McGurk kindly provided us values of the relationship he presents for 199 populations (196 provided) of pelagic marine fish eggs and larvae, juvenile and adult marine fishes, whales, and marine pelagic invertebrates. Our first question concerns the validity of pooling data from such diverse groups. Comparison of slopes among these groups (Table 1) indicates that there is a significant difference between slopes of different categories as grouped by Mr. McGurk (analysis of covariance $P(F = 2.65) < 0.05$). When the data are subdivided into logical groupings (Table 1), slopes for the weight mortality relationship are quite variable (−0.91 to 0.42).

TABLE 1. Comparison of slopes of the weight−mortality relationship between different taxa and groupings for data supplied by Michael McGurk

McGurk's categories	r^2	N	Ordinary slope	GM slope
Pelagic marine fish eggs and larvae	0.38	72	−0.54	−0.88
Juvenile and adult marine fishes	0.54	94	−0.34	−0.46
Whales	0.35	7	0.42	0.70
Marine pelagic invertebrates	0.06	23	−0.16	−0.68
Other possible categories				
Fish eggs	0.65	30	−0.91	−1.13
Fish larvae	0.22	42	−0.34	−0.73
Juvenile and adult fish (5−2900 g)	0.41	67	−0.37	−0.40

Another question concerns whether an ordinary predictive regression or geometric mean (GM) regression is the best descriptor of the data (Ricker 1973, 1975, 1984). Given the mutual uncertainty of variables and somewhat artificial nature of McGurk's data set, the GM regression may be more acceptable (expanding the range of slopes to −1.13 to 0.70). When examined within categories, it is notable that fish eggs and larvae have a much more negative slope than other categories, that whales have a positive slope, and pelagic invertebrates do not have a significant relationship between size and mortality rate ($P(F = 1.27) > 0.25$). Accepting that pooling these diverse taxa is not really justified on statistical grounds, we will restrict our examination to values for juvenile and adult fish between the size limits we give for Babine Lake sockeye salmon (5.35−2919 g wet weight). Mr. McGurk's data suggest a relationship with a slope of -0.584 ± 0.111 (95% C.I. GM regression) or -0.374 ± 0.111 (95% C.I. ordinary predictive regression). Neither of these values agrees with Mr. McGurk's slope of −0.25 nor with ours of −1.00 and we hesitate to judge which slope is the better estimate.

The data points provided by Mr. McGurk are derived in numerous ways. Mortality rates are taken from catch curves of virgin populations, mark−recapture methods, and regressions of total mortality on effort, as well as other methods. Weights

are derived as the average length over which the mortality estimate was made, the definite integral of a length age equation, or the midpoint of the length range. These give a single mortality rate for an average population member at a single size. This approach may tend to obscure the very concept we have tried to examine: that within a species, mortality rates decrease as the fish grow and a single mortality rate estimate does not sufficiently describe the species' population dynamics. We believe that a far more satisfactory approach would be to examine in detail the mortality schedule of individual species throughout their life history and then make comparisons between the different species.

From a biological perspective, this data set includes species of both pelagic and benthic habits from coastal and oceanic areas. Some species alter their habitat through ontogeny. A single weight mortality factor applied to such a species mixture would be surprising and, if true, of considerable significance. It would imply that the many biotic and abiotic agents of natural mortality acted in a complementary manner to yield identical results, relative to size, for all fish species, at all life history stages in all habitats. Including whales and invertebrates, as in Mr. McGurk's fig. 1, suggests that this generality encompasses metabolically distinct homeotherms and heterotherms.

In our presentation we deal with sockeye salmon from their entry into the ocean until they return to their native river mouths as adults. In the first month at sea the fish undergo a physiological stress changing from freshwater to marine systems. The smolt's arrival in estuaries is heralded by a host of aggregated predators. The survivors move gradually offshore into a pelagic environment with fewer predators and return to the coastal area as one of the terminal predators in the food web. Considering the diversity of habitats through which sockeye salmon grow, it is possible a single weight mortality factor may not apply throughout. Our use of this approach was for two reasons. It allowed comparison, in a more complex analysis, with estimates produced by other authors using the same factor (Mathews and Buckley 1976; Ricker 1976) and it permitted development of a method to make these estimates applicable to all the sockeye salmon's life history types in a single cohort. Factors other than −1.0 could easily be incorporated into our model. We did not examine them in our sensitivity analysis because none had been specifically proposed for sockeye or other salmon and we did not feel satisfied that a factor could be derived from ethologically and taxonomically remote species. Factors derived from other species followed from juvenile to adult may be more reliable than a relationship derived by selecting a single mortality rate and size for a variety of species. Fortunately there appears to be growing interest in this topic and useful data such as those compiled by Mr. McGurk will likely aid in understanding the general relationship between size and mortality — Donald J. Furnell and J. R. Brett, *Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.*
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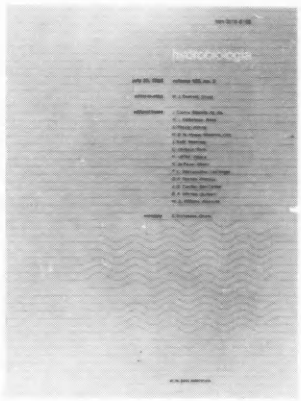

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A General Multivariate Dose–Response Model

J. Schnute and J. O. T. Jensen

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

Schnute, J., and J. O. T. Jensen. 1986. A general multivariate dose–response model. Can. J. Fish. Aquat. Sci. 43: 1684–1693.

This paper presents a general model for describing and predicting biological response to environmental factors. The theory embraces concepts and models used in earlier studies of the effects of toxicity on fish, and it extends these ideas in several directions. It can include an arbitrary number of factors, either detrimental or beneficial to the organism. Also, its statistical formulation allows (1) hypothesis testing to determine the relative importance of various factors and (2) calculation of critical factor levels that (with prescribed confidence) assure a community response no worse than a specified level. Our model is based on earlier work by Schnute and McKinnell, and, like theirs, its parameters have biologically meaningful interpretations. Our discussion includes an explanation of how the model relates specifically to toxicity studies. In a companion paper (Can. J. Fish. Aquat. Sci. 43: 1694–1709), we use our model to investigate salmonid response to gas supersaturation and ancillary factors.

Les auteurs présentent un modèle général de description et de prévision de la réponse biologique à des facteurs environnementaux. La théorie englobe des concepts et des modèles utilisés au cours d'études antérieures portant sur les effets de la toxicité chez le poisson et les développent dans plusieurs directions. Le modèle peut comprendre un nombre arbitraire de facteurs à effets nocifs ou utiles pour l'organisme. Sa formulation statistique permet aussi 1) d'effectuer un test d'hypothèse visant à déterminer l'importance relative de divers facteurs et 2) de calculer des niveaux critiques (à un niveau de confiance établi) garantissant que la réponse d'une communauté ne sera pas inférieure à un niveau déjà précisé. Le modèle repose sur des travaux antérieurement effectués par Schnute et McKinnell et, comme pour ces travaux, ses paramètres autorisent des interprétations biologiquement significatives. Les auteurs traitent de la façon dont le modèle peut être spécifiquement appliqué à des études de toxicité. Ils traitent aussi dans une publication connexe (Can. J. Fish. Aquat. Sci. 43: 1694–1709) de l'application du modèle à la réponse de salmonidés à une sursaturation gazeuse et à d'autres facteurs connexes.

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A biologist concerned with pollution invariably faces the problem of describing community response to toxicant levels. For example, a common measure of response is the exposure time to 50% mortality (ET50). Typical questions to be resolved include the following: (1) What toxic level causes the response to drop below a prescribed threshhold? (2) What is the combined effect of several toxicants? (3) Are toxic effects altered by changes in environmental variables, such as temperature? (4) Does toxicity depend on characteristics of the organism, such as animal size?

The resolution of such problems requires a model relating the response y to various factors $x_1, x_2, \ldots$. These factors may represent toxicant levels, environmental variables, organism characteristics, and so on. For example, if y represents ET50 and x measures a lethal toxicant concentration, then y is a decreasing function of x; that is, the greater the toxic level x, the shorter the time y required for half the population to die. Three equations used historically to describe this relationship are

$$\log y = a - b \log x, \tag{1.1}$$

implicit in Sprague (1969) and Knittel et al. (1980),

$$y = -c \log (a - bx^{-1}), \tag{1.2}$$

proposed by Zitko (1979, 1982), and

$$(y - a)(x - b)^c = d, \tag{1.3}$$

suggested in a diverse literature, including Wuhrmann and Woker (1950), Wuhrmann (1952), and Warren and Doudoroff (1971). In (1.1)–(1.3), a, b, c, and d represent constants, not necessarily comparable among the equations. These equations exhibit certain similarities; for example, (1.3) includes a log–log relationship like (1.1) as a special case.

The main feature of models (1.1)–(1.3) is that they describe a monotonic (increasing or decreasing) relationship between x and y. Notice that, depending on the signs of the constants, equations (1.1)–(1.3) allow for a factor that either favors or inhibits the response; that is, increasing x must cause y either to decrease or to increase steadily. This paper presents a model that preserves this general idea, but extends it in several directions. First, our model can include an arbitrary number of factors. Second, the possible relationships between any one factor x and y can be enumerated precisely. For example, a factor may favor y up to a certain level at which the beneficial gain stops; or it may cause y to decrease, but not below a minimum value. A full set of 16 possibilities is presented later. Third, the model parameters allow for a quick judgement as to the relative importance of the factors. Fourth, the model is

rendered statistical, so that hypothesis tests can be used to assess the importance of factors and to add or remove them in a manner analogous to stepwise regression. Finally, all necessary equations for confidence levels are derived so that, for example, one can answer the following question: At what level of factor x is it 95% certain that the response y will be greater than some prescribed response y_0?

The model proposed here is based on earlier work by Schnute and McKinnell (1984). They were concerned with an optimal (minimum or maximum) response for some set of factor levels, whereas the model here is simpler, since it is monotone in the factors. All that is required for the simplification is to drop the quadratic terms in transformed values of the factors. The purpose of this paper is to explain the significance of the model in the context of dose–response relationships, to improve Schnute and McKinnell's (1984) analysis of the various cases of the model, and to derive the confidence level formulas required for finding toxicity tolerance limits.

In a companion paper (Jensen et al. 1986), we apply our model to literature data on salmonid response to gas supersaturation and associated ancillary factors. We also describe techniques of exploratory data analysis for making a preliminary assessment of the model's suitability.

The Model

To state the model precisely, let y be the response to the m factors $x_1, \ldots, x_m$. Let u_i and U_i be typical low and high values, respectively, for the factors x_i $(i = 1, \ldots, m)$; similarly, let v and V be typical low and high values of the response y. Then the model considered here is defined by the following three equations:

$$\eta = p + \sum_{i=1}^{m} q_i \xi_i \tag{2.1}$$

$$\xi_i = (2x_i^{\alpha_i} - U_i^{\alpha_i} - u_i^{\alpha_i})/(U_i^{\alpha_i} - u_i^{\alpha_i}) \tag{2.2*}$$

$$\eta = (2y^{\gamma} - V^{\gamma} - v^{\gamma})/(V^{\gamma} - v^{\gamma}). \tag{2.3*}$$

In (2.2) and (2.3), the values $u_1, \ldots, u_m, U_1, \ldots, U_m, v$, and V are prescribed in advance by the user to conform to reasonable limits for the data. Thus, these are presumed to be *known* quantities, in contrast with the $2m + 2$ unknown parameters: $m + 1$ coefficients $p, q_1, \ldots, q_m$, and $m + 1$ exponents $\alpha_1, \ldots, \alpha_m, \gamma$. The new variables ξ_i and η are dimensionless counterparts of the original x_i and y, respectively, scaled between -1 and 1 for typical low and high values of the original variables. The model itself, equation (2.1), defines a linear relationship between η and ξ_i, that is, between the transformed value of the response and transformed values of the factors. As described in the introduction, this is the special case of Schnute and McKinnell's (1984) response surface obtained by setting the quadratic coefficients (labelled r_{ij} by Schnute and McKinnell) equal to 0.

To see precisely how the model (2.1)–(2.3) defines the response in terms of the factors, consider the back-transformed version of (2.3),

$$y = \left[\frac{V^{\gamma} + v^{\gamma} + (V^{\gamma} - v^{\gamma})\eta}{2}\right]^{1/\gamma}, \tag{2.4*}$$

which expresses y in terms of η. Given the factors x_i $(i = 1, \ldots, m)$, one can compute the transforms ξ_i $(i = 1, \ldots, m)$ from (2.2). These, in turn, determine η from (2.1), and η determines y from the back-transform (2.4). Incidentally, it may happen that the quantity in square parentheses in (2.4) is negative, so that y is not defined. Although this indicates factor levels outside the range of model validity, it is convenient numerically to adopt the convention in (2.4) that

$$y = -(-Q)^{1/\gamma}$$

when $Q < 0$, where Q is the quantity in square parentheses. This convention avoids computing errors and yet indicates invalid y values by reporting $y < 0$.

The model coefficients p and q_i $(i = 1, \ldots, m)$ typically have magnitude around 1, since they relate quantities in (2.1) with this order of magnitude. If any q_i is small, then the corresponding x_i has little influence on y within the range of the data. An increase in x_i causes y to increase or decrease when $q_i > 0$ or $q_i < 0$, respectively. The exponents γ and α_i $(i = 1, \ldots, m)$ relate to the nonlinear nature of the response. The factor x_i has greatest influence on y at low levels of x_i when $\alpha_i < 1$ and high levels of x_i when $\alpha_i > 1$. Similarly, the combined effect of all factors is most influential at low values of y when $\gamma > 1$ and high values of y when $\gamma < 1$.

Notice that the model includes two parameters for each factor x_i: the coefficient q_i (associated with the factor's influence) and the exponent α_i (associated with a transformation to account for nonlinearity). In this sense, the model is parsimonious. When $\alpha_i = 1$, the model is linear in x_i, and q_i is the usual regression coefficient. Obviously, it is impossible to include a factor without introducing a coefficient like q_i. In the present context we also need a second parameter, like α_i, to account for an unknown nonlinear effect on the response. The reason for the rather complex formulation (2.1)–(2.3), involving the specified quantities u_i, U_i, v, and V, is that the resulting parameters q_i and α_i tend to be statistically independent in practice. Thus, for example, it may turn out that (for some i) q_i is well determined, although α_i is not. If so, we can say with some confidence that the factor x_i is influential, but we are less certain about the appropriate transformation to account for nonlinearity. Our companion paper (Jensen et al. 1986) illustrates this possibility.

The present paper follows a convention adopted by Schnute and McKinnel (1984) that equations such as (2.2) and (2.3) with continuous extensions to zero exponents are marked with an asterisk. For example, as γ tends to 0 in (2.3), the transformation takes the limiting form

$$\eta = \frac{\log\,[y^2/(Vv)]}{\log\,(V/v)}. \tag{2.5}$$

Thus, the case $\gamma = 0$ essentially corresponds to a log transform of y. It is particularly interesting to compare the cases $\gamma = 1$ and $\gamma = 0$. When $\gamma = 1$, the factors x_i (through their transforms ξ_i) influence y additively in (2.1). By contrast, when $\gamma = 0$, the factors influence log y additively; that is, they influence the response y itself multiplicatively.

In many applications, y represents ET50, the exposure time to 50% mortality. The response $y = \infty$, if it can be extrapolated from the data, holds particular interest because it implies that the organisms do not die; that is, the corresponding factor levels are not lethal. When $\gamma < 0$, the model admits this possibility. As above, let Q represent the quantity in square brackets in (2.4). Notice that, with $\gamma < 0$, y becomes increasingly large as Q becomes smaller. Finally, $y = \infty$ when $Q = 0$, that is, when

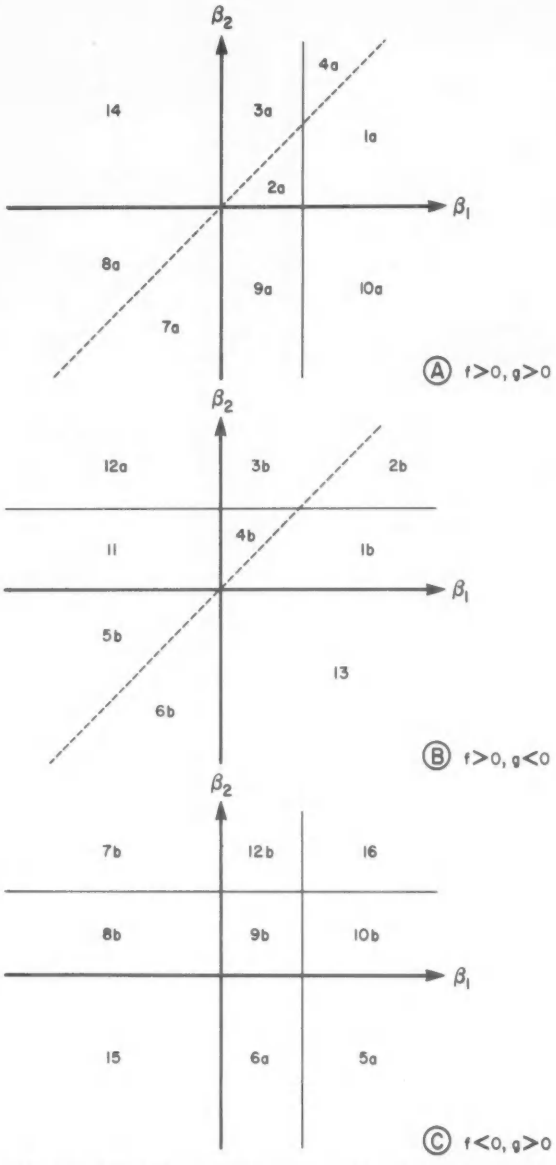

FIG. 1. Regions in the space of parameters (f, g, β_1, β_2) for equation (2.9). In panels A and B the broken line is characterized by $\beta_1 = \beta_2$. In panels A and C the lines parallel to the β_2-axis have $\beta_1 = 1$, and in panels B and C the lines parallel to the β_1-axis have $\beta_2 = 1$. The values of β_1 and β_2, together with the signs of f and g, determine a particular region 1a, 1b, 2a, 2b, . . . , 16. This region, in turn, determines a curve type from Fig. 2 and 3.

$$\eta = \frac{v^\gamma + V^\gamma}{v^\gamma - V^\gamma}. \tag{2.6}$$

Combining (2.1) and (2.6) gives the condition

$$p + \sum_{i=1}^{m} q_i \xi_i = \frac{v^\gamma + V^\gamma}{v^\gamma - V^\gamma} \tag{2.7}$$

on the factors x_i, through their transforms ξ_i. Thus, when $\gamma < 0$, (2.7) defines the boundary where $y = \infty$. In the present context, the factors theoretically cause mortality on one side of this boundary and are not lethal on the other side. In particular, with only one factor ($m = 1$), it follows from (2.7) and (2.2) that the level of x_1 where $y = \infty$ is given by

$$x = \left[\frac{u^\alpha + U^\alpha}{2} - \frac{U^\alpha - u^\alpha}{2q(V^\gamma - v^\gamma)} \times [(1 + p)V^\gamma + (1 - p)v^\gamma]\right]^{1/\alpha} \tag{2.8}$$

where subscripts "1" have been dropped throughout.

To understand the geometric significance of the model (2.1)−(2.3), it is useful to consider the equation

$$z_2^{\beta_2} = f z_1^{\beta_1} + g, \tag{2.9}$$

which involves two variables z_1 and z_2, two exponents β_1 and β_2, and two coefficients f and g. Depending on the values of f, g, β_1, and β_2, the graph of z_2 versus z_1 in (2.9) can take various forms. Since both z_1 and z_2 must be positive, it follows that f and g cannot both be negative. This leaves three possible cases: (A) $f > 0$ and $g > 0$, (B) $f > 0$ and $g < 0$, and (C) $f < 0$ and $g > 0$. To determine the shape of a curve (2.9), it is enough to know only the exponents β_1 and β_2 and the case (A), (B), or (C). Figures 1, 2, and 3 present the possible shapes systematically. First, from Fig. 1, the case and the exponents determine a particular region in the $\beta_1\beta_2$ plane, labelled 1a, 1b, 2a, etc. The label then determines a curve type in Fig. 2 or 3, where the letter "a" or "b" typically distinguishes two possible intercepts of the curve. Mathematical proofs underlying Fig. 1, 2, and 3 are given in Appendix A. These results correspond to earlier work by Schnute and McKinnell (1984), but the formulation here differs from theirs and is more convenient to apply, particularly in the present context.

Several general features of the curve shapes have particular interest. To give the ideas a specific context, suppose that z_2 is the exposure time to 50% mortality (ET50) in the presence of a factor z_1. Then, significant features of Fig. 1, 2, and 3 include the following.

(1) The curves either increase (types 1−8, Fig. 2) or decrease (types 9−16, Fig. 3) steadily as functions of z_1. A curve increases when $\beta_1\beta_2 f > 0$ and decreases when $\beta_1\beta_2 f < 0$. Thus, the sign of $\beta_1\beta_2 f$ (+ or −, respectively) determines whether the factor z_1 is favorable or detrimental to survival.

(2) Curves in case (A) are defined for all values of z_1 and yet do not take all possible values of z_2. Type 9a illustrates this feature; here, there is a finite time to mortality, such as a natural life span, even when z_1 is absent ($z_1 = 0$); but as the detrimental factor z_1 increases, this time is reduced almost to 0 (immediate death). Type 1a exhibits a similar feature when z_1 is favorable. Again, there is a finite time to mortality when $z_1 = 0$, but this is increased at an accelerating rate as z_1 increases.

(3) Curves in case (B) are defined only for some values of z_1 and yet take all possible values of z_2. For example, consider type 13. Here, as in the discussion leading to (2.8), there is a critical level of z_1 below which mortality does not occur. Above this level, the time to mortality decreases steadily as z_1 increases, until death is almost immediate. Types 5b and 6b illustrate a related feature when z_1 is favorable. In these cases, there is instant death ($z_2 = 0$) when z_1 is absent ($z_1 = 0$); as z_1 increases, z_2 increases rapidly until, at a critical value of z_1, there is no further mortality. Thus, in types 5b and 6b, z_1 might

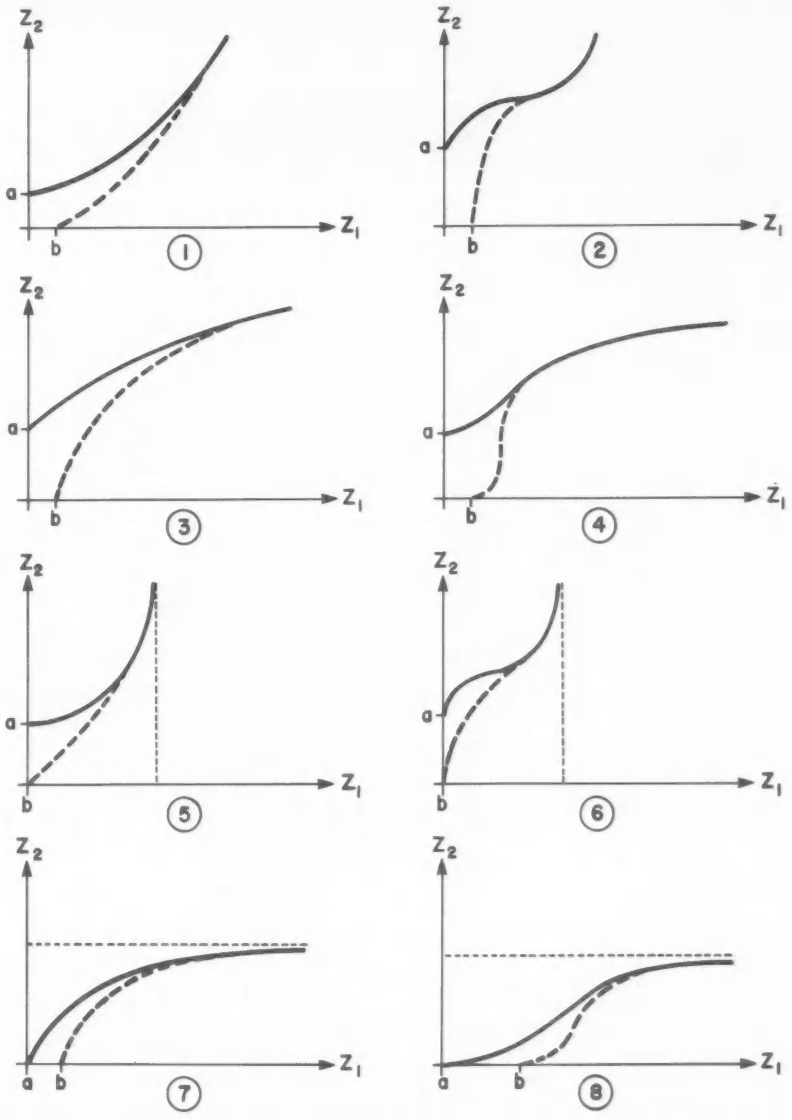

FIG. 2. Curve types determined by equation (2.9) when $\beta_1\beta_2 f > 0$. These have the common feature that z_2 is an increasing function of z_1. The particular type 1a, 1b, 2a, 2b, . . . , 8 corresponds to the region in Fig. 1 indicated by the parameters in (2.9).

represent availability of a nutrient: a certain amount is essential for life, but beyond a threshold where the requirement is saturated, no more is necessary.

(4) Curves in case (C) are defined for only some values of z_1 and take only some values of z_2. Type 15 illustrates this possibility. Here, as in type 13, there is a critical level of z_1 below which mortality does not occur. Above this level, the time to mortality decreases, but only down to a finite limit, as z_1 increases.

(5) Curves may or may not have inflection points. For example, compare 3a and 4a. In 3a, z_1 has a steadily decelerating favorable influence on z_2, while in 4a, the effect of z_1 accelerates at first and then ultimately decelerates. Mathematical formulas for inflection points, when they exist, are given in Appendix A.

The above examples are based on interpreting (2.9) as a dose–response relationship. Of course, (2.9) has many other possible biological interpretations. For example, the reader might relate types 7 and 8 to animal growth with an asymptotic size limit. In the present context, these same curve types might indicate a favorable factor z_1 which cannot, however, increase the time to mortality z_2 beyond some ultimate limit, such as a natural life span.

The curves (2.9) relate to the model (2.1)–(2.3) in at least two ways. First, they represent the response y when there is only one factor x_1. In this case, (2.9) applies with $z_1 = x_1$,

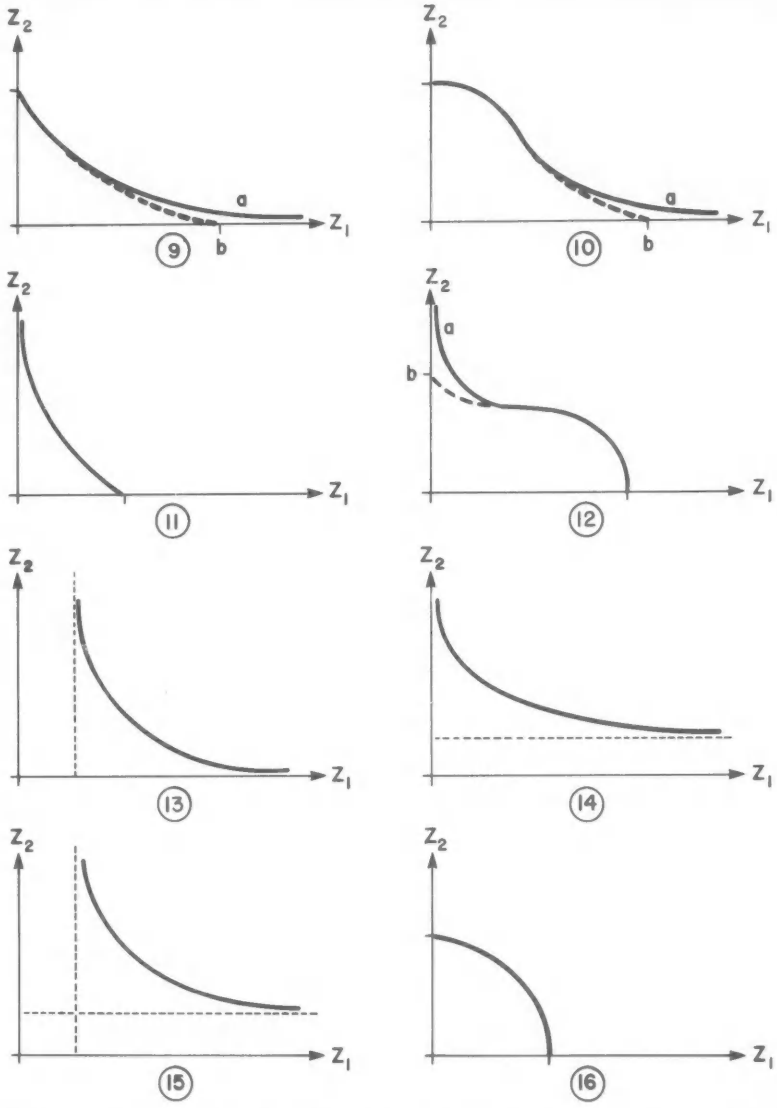

FIG. 3. Curve types determined by equation (2.9) when $\beta_1 \beta_2 f < 0$. These have the common feature that z_2 is a decreasing function of z_1. The particular type 9a, 9b, 10a, 10b, . . . , 16 corresponds to the region in Fig. 1 indicated by the parameters in (2.9).

$z_2 = y$, $\beta_1 = \alpha_1$, $\beta_2 = \gamma$, and f and g given sequentially by

$$f = q_1(V^\gamma - v^\gamma)/(U_1^{\alpha_1} - u_1^{\alpha_1}) \tag{2.10}$$

$$g = \tfrac{1}{2}[(1+p)V^\gamma + (1-p)v^\gamma - f(U_1^{\alpha_1} + u_1^{\alpha_1})]. \tag{2.11}$$

Second, the curves (2.9) also represent a contour of (2.1)–(2.3) when there are two factors ($m = 2$). Let y_0 be the value of y on a particular contour, and let η_0 be the corresponding value of η from (2.3). Then, the contour is described by (2.9) with $z_i = x_i$ ($i = 1,2$), $\beta_1 = \alpha_i$ ($i = 1,2$), and f and g given sequentially by

$$f = -(q_1/q_2)(U_2^{\alpha_2} - u_2^{\alpha_2})/(U_1^{\alpha_1} - u_1^{\alpha_1}) \tag{2.12}$$

$$g = \tfrac{1}{2}\left[\frac{\eta_0 - p}{q_2}(U_2^{\alpha_2} - u_2^{\alpha_2}) + f(U_1^{\alpha_1} + u_1^{\alpha_1}) + (U_2^{\alpha_2} + u_2^{\alpha_2})\right]. \tag{2.13}$$

More generally, (2.9) can represent the general case of m factors either as (1) the response function when all factors but one are fixed or (2) a response contour when both y and all factors but two are fixed.

We can compare our model to the following generalization of (1.3):

$$(2.14) \quad \log (y - a) = \log d - \sum_{i=1}^{m} c_i \log (x_i - b_i).$$

Like ours, model (2.14) involves $2m + 2$ parameters: $a_1, \ldots, a_m, b_1, \ldots, b_m, c$ and d. When $c_i > 0$ in (2.14), a and b_i correspond to asymptotic values for the response y and the factor x_i. Curve type 15 (Fig. 3) shows that our model also allows for such asymptotes without imposing the requirement in (2.14) that the variables are log transformed. For example, (2.14) allows *only* a multiplicative effect of the factors, while our model includes the case ($\gamma = 1$) that factors influence the response additively. Our philosophy is to allow the data to speak for themselves in selecting the appropriate transformation.

The model (2.1)−(2.3) is designed primarily for a response y which, like ET50, can take arbitrary positive values from 0 to ∞. Sometimes, however, bioassay involves studying the fraction y of organisms that survive a prescribed set of deleterious conditions. In such cases, the response y is necessarily restricted to lie between 0 and 1. The transformation (2.3) may then be inappropriate, and a better choice might be

$$(2.15^*) \quad \eta = \frac{\log \{(y^\gamma - 1)^2 / [(V^\gamma - 1)(v^\gamma - 1)]\}}{\log \{(V^\gamma - 1)/(v^\gamma - 1)\}}$$

where v and V (typical low and high values of y) are restricted to lie between 0 and 1. The transformation (2.15), originally suggested by Schnute and McKinnell (1984), generalizes the logistic transform commonly applied in bioassay. When $\gamma = -1$, (2.15) is logistic and, consequently, symmetric with respect to the inflection point at $y = 0.5$. The extra parameter γ in (2.15) allows for asymmetry around the inflection point. The inverse of this transform is

$$(2.16^*) \quad y = [1 - \text{sign}(\gamma) |1 - V^\gamma|^{(1+\eta)/2} \times |1 - v^\gamma|^{(1-\eta)/2}]^{1/\gamma}.$$

Thus, (2.15)−(2.16) can be regarded as counterparts to (2.3)−(2.4) when the response y is restricted to the range 0 to 1. In this sense, the classical theory of logistic bioassay can readily be generalized along the lines developed here by using the model (2.1), (2.2), and (2.15) in place of (2.1)−(2.3). Because we are concerned primarily with responses y, like ET50, that can take arbitrary positive values, we restrict our mathematical development to the model (2.1)−(2.3).

Likelihood and Hypothesis Tests

Biological data, of course, never precisely conform to the model (2.1)−(2.3). Schnute and McKinnell (1984) described a systematic way to introduce statistical error. This section summarizes their results as applied to the model here. Suppose that there are n data points with the observed response $\hat{y}_j$ corresponding to the factor vector

$$(3.1) \quad \boldsymbol{X}_j = (x_{1j}, x_{2j}, \ldots, x_{mj})$$

where $j = 1, \ldots, n$. Let $\hat{\eta}_j$ be the transformed value of $\hat{y}_j$ from (2.3); similarly, let η_j and y_j be model predictions from the factor vector $\boldsymbol{X}_j$. Then the error assumption is that

$$(3.2) \quad \hat{\eta}_j = \eta_j + \sigma \epsilon_j$$

where σ is the standard deviation of $\hat{\eta}_j$ and the model errors $\epsilon_1, \ldots, \epsilon_n$ are independent and normal with mean 0 and variance 1. From (3.2), it can be shown that the error in $\hat{y}$ depends on γ; for example, the error is normal when $\gamma = 1$ and log-normal when $\gamma = 0$.

The model (2.1)−(2.3) involves the parameter vector

$$(3.3) \quad \boldsymbol{\theta} = (p, q_1, \ldots, q_m, \alpha_1, \ldots, \alpha_m, \gamma)$$

of coefficients and exponents, and the prediction $y_j(\boldsymbol{\theta})$ at $\boldsymbol{X}_j$ depends on $\boldsymbol{\theta}$. Schnute and McKinnell (1984) showed that the maximum likelihood estimate $\hat{\boldsymbol{\theta}}$ for $\boldsymbol{\theta}$ can be obtained by minimizing the function

$$(3.4^*) \quad A(\boldsymbol{\theta}) = \sum_{j=1}^{n} \left[\frac{\hat{y}_j^\gamma - y_j(\boldsymbol{\theta})^\gamma}{\gamma Y^{\gamma - 1}} \right]^2$$

where Y is the geometric mean of the observed responses, that is,

$$Y = \left[\prod_{j=1}^{n} \hat{y}_j \right]^{1/n}$$

Once $\hat{\boldsymbol{\theta}}$ is known, then a bias-corrected likelihood estimate for σ turns out to be determined by

$$(3.5^*) \quad \hat{\sigma}^2 = \frac{1}{n - k} \sum_{j=1}^{n} \left[\frac{\hat{y}_j^{\hat{\gamma}} - y_j(\hat{\boldsymbol{\theta}})^{\hat{\gamma}}}{V^{\hat{\gamma}} - v^{\hat{\gamma}}} \right]^2$$

where k is the number of parameters that have been estimated. Schnute and McKinnell (1984) described an efficient method for minimizing $A(\boldsymbol{\theta})$ to obtain $\hat{\boldsymbol{\theta}}$ (see also Mittertreiner and Schnute 1985, p. 54).

We frequently must ask whether or not the addition or removal of a factor is statistically significant in determining the response. Essentially, this question amounts to a hypothesis test on $\boldsymbol{\theta}$. For example, if $m = 3$ and the third factor x_3 is unimportant, then we should not be able to reject the hypothesis $q_3 = 0$ against the alternative $q_3 \neq 0$. In general, suppose that $\boldsymbol{\theta}$ is written in two parts:

$$\boldsymbol{\theta} = (\boldsymbol{\theta}_k, \boldsymbol{\theta}_l)$$

where $\boldsymbol{\theta}_k$ and $\boldsymbol{\theta}_l$ have k and l components, respectively. Suppose we wish to test the null hypothesis

$$H_0: \boldsymbol{\theta}_k = \boldsymbol{\theta}_k^*$$

with prescribed $\boldsymbol{\theta}_k^*$ against the alternative

$$H_1: \boldsymbol{\theta}_k \neq \boldsymbol{\theta}_k^*.$$

Let A_0 and A_1 represent the minima of A under H_0 and H_1, respectively. Then, if H_0 is true, the statistic

$$(3.6) \quad B = \frac{n - k - l}{k} \frac{A_0 - A_1}{A_1}$$

is approximately F-distributed with k and $n - k - l$ df. Thus if B exceeds, say, the upper 95% point for F, then H_0 can be rejected with 95% confidence. This test is approximate, in general, because the model involves some parameters (namely the exponents) nonlinearly. Consequently, the test should be regarded informally, more as a guide for understanding than a rigorous statement of statistical probabilities.

Variances and Confidence Limits

A full application of the model (2.1)−(2.3) typically involves finding confidence intervals for the parameter estimates and model predictions. For example, suppose that y represents ET50 and $\boldsymbol{X}$ represents a vector of toxicant concentrations. Let $y(\boldsymbol{X})$ denote the model's predicted ET50 based on the value of

$\boldsymbol{X}$. An actual measured response $\hat{y}$ in the presence of toxicity levels $\boldsymbol{X}$ will typically be either higher or lower than this prediction. The biologist is particularly concerned with instances when the observed $\hat{y}$ is less than the predicted $y(\boldsymbol{X})$; that is, the observed survival time to 50% mortality is shorter than predicted. It is clearly important to be able to compute a lower limit $y'(\boldsymbol{X})$ such that, given $\boldsymbol{X}$, an observed $\hat{y}$ is guaranteed to be higher than $y'(\boldsymbol{X})$ at least, say, 95% of the time. In fact, it could be argued that the lower limit $y'(\boldsymbol{X})$ is actually more important than the prediction $y(\boldsymbol{X})$ for the purposes of environmental management.

The calculations required to find a confidence limit like $y'(\boldsymbol{X})$ are somewhat extensive and require a suitable notation. Consistent with (3.3), let $\boldsymbol{\theta}$ be the parameter vector

$$\boldsymbol{\theta} = (\theta_1, \ldots, \theta_{2m+2}) \tag{4.1}$$

where

$$\theta_1 = p$$
$$\theta_{i+1} = q_i;\ i = 1, \ldots, m$$
$$\theta_{i+m+1} = \alpha_i;\ i = 1, \ldots, m$$
$$\theta_{2m+2} = \gamma.$$

Define the function

$$F(\boldsymbol{X}, y, \boldsymbol{\theta}) = \eta - \sum_{i=1}^{m} q_i \xi_i \tag{4.2}$$

where, as usual in this paper,

$$\boldsymbol{X} = (x_1, \ldots, x_m)$$

is a vector of factors with the transformed coordinates ξ_i from (2.2) and y is a response with the transformed value η from (2.3). Then, the model (2.1)−(2.3) simply boils down to the assertion that

$$F(\boldsymbol{X}, y, \boldsymbol{\theta}) = 0. \tag{4.3}$$

The derivatives of F with respect to the parameters are required for calculating variances. For $k = 1, \ldots, 2m + 2$, define

$$G_k = \partial F / \partial \theta_k. \tag{4.4}$$

Then, it turns out that

$$G_1 = -1 \tag{4.5}$$

$$G_{2m+2} = \frac{2y^\gamma \log y - (1 + \eta) V^\gamma \log V - (1 - \eta) v^\gamma \log v}{V^\gamma - v^\gamma} \tag{4.6}$$

and, for $i = 1, \ldots, m$,

$$G_{i+1} = -\xi_i \tag{4.7}$$

$$G_{i+m+1} = -q_i \frac{2x_i^{\alpha_i} \log x_i - (1 + \xi_i) U_i^{\alpha_i} \log U_i - (1 - \xi_i) u_i^{\alpha_i} \log u_i}{U_i^{\alpha_i} - u_1^{\alpha_i}} \tag{4.8}$$

Conceptually, each derivative G_k is a function of $\boldsymbol{X}$, y, and $\boldsymbol{\theta}$:

$$G_k = G_k(\boldsymbol{X}, y, \boldsymbol{\theta}),$$

although typically a derivative depends on only one or two coordinates of the vectors $\boldsymbol{X}$ and $\boldsymbol{\theta}$. For example, G_{i+m+1} depends only on x_i, q_i, and α_i. Incidentally, notice that η and ξ_i in (4.6)−(4.8) refer to the usual transforms computed from y, γ, x_i, and α_i.

As in the preceding section, suppose that n observations $(\boldsymbol{X}_j, \hat{y}_j)$, $j = 1, \ldots, n$, have been made and that $\hat{\boldsymbol{\theta}}$ is the estimated parameter vector for these data. Then define

$$H_{kl} = \sum_{j=1}^{n} G_k(\boldsymbol{X}_j, \hat{y}_j, \hat{\boldsymbol{\theta}})\, G_l(\boldsymbol{X}_j, \hat{y}_j, \hat{\boldsymbol{\theta}}) \tag{4.9}$$

for $k = 1, \ldots, N$ and $l = 1, \ldots, N$, where

$$N = 2m + 2$$

is the number of parameters (the dimension of $\boldsymbol{\theta}$). Also, let

$$S = \sum_{j=1}^{n} [F(\boldsymbol{X}_j, \hat{y}_j, \hat{\boldsymbol{\theta}})]^2. \tag{4.10}$$

Then the elements H_{kl} define a symmetric matrix $\boldsymbol{H}$ from which the matrix

$$\boldsymbol{C} = \frac{S}{n - N} \boldsymbol{H}^{-1} \tag{4.11}$$

can be defined, where $\boldsymbol{H}^{-1}$ represents the matrix inverse of $\boldsymbol{H}$.

It turns out that $\boldsymbol{C}$ is an estimate of the covariance matrix for the components of the estimated parameter vector $\hat{\boldsymbol{\theta}}$; that is, if C_{kl} is a component of $\boldsymbol{C}$, then it is approximately true that

$$\mathrm{Cov}(\hat{\theta}_k, \hat{\theta}_l) = C_{kl}. \tag{4.12}$$

From a practical point of view, uncertainty in the model's parameters is perhaps less important than uncertainty in its predictions. To describe the error in prediction, we first need a notation that distinguishes several related quantities. As above, let $\hat{\boldsymbol{\theta}}$ be the parameter vector estimated from a data set. This differs, in general, from the unknown true parameter vector $\boldsymbol{\theta}^*$. Suppose that a factor vector $\boldsymbol{X}$ is given. Define

$$\bar{y} = \bar{y}(\boldsymbol{X}, \hat{\boldsymbol{\theta}})$$

to be the predicted response to $\boldsymbol{X}$ based on the parameter estimate $\hat{\boldsymbol{\theta}}$. Similarly, let

$$y^* = y^*(\boldsymbol{X}, \boldsymbol{\theta}^*)$$

be the correct prediction based on the true parameter vector $\boldsymbol{\theta}^*$. Finally, let

$$\hat{y} = \hat{y}(\boldsymbol{X}, \boldsymbol{\theta}^*, \epsilon)$$

denote a new observed response associated with the factor level $\boldsymbol{X}$, the true parameter vector $\boldsymbol{\theta}^*$, and a random error ϵ as in (3.2). Notice that $\bar{y}$, y^*, and $\hat{y}$ are all different, in general. The known prediction $\bar{y}$ differs from the unknown true prediction y^* due to the error in the parameter estimate $\hat{\boldsymbol{\theta}}$. The observation

$\hat{y}$ differs from the known prediction $\tilde{y}$ because, first, $\tilde{y}$ contains the error due to the estimate $\hat{\boldsymbol{\theta}}$ just described and, second, $\hat{y}$ includes the random error ϵ.

To quantify these errors, define the following two functions of $\boldsymbol{X}$:

$$(4.13) \quad D(\boldsymbol{X}) = \sum_{k=1}^{m} \sum_{l=1}^{m} G_k(\boldsymbol{X}, \tilde{y}, \hat{\boldsymbol{\theta}})\, G_l(\boldsymbol{X}, \tilde{y}, \hat{\boldsymbol{\theta}})\, C_{kl}$$

$$(4.14) \quad E(\boldsymbol{X}) = D(\boldsymbol{X}) + \frac{S}{n-N}.$$

Then it is approximately true that

$$(4.15) \quad \frac{2(y^{*\hat{\gamma}} - \tilde{y}^{\hat{\gamma}})}{\sqrt{D(\boldsymbol{X})}\,(V^{\hat{\gamma}} - v^{\hat{\gamma}})} \sim t(n-N)$$

$$(4.16) \quad \frac{2(\hat{y}^{\hat{\gamma}} - \tilde{y}^{\hat{\gamma}})}{\sqrt{E(\boldsymbol{X})}\,(V^{\hat{\gamma}} - v^{\hat{\gamma}})} \sim t(n-N)$$

where $\hat{\gamma}$ is the estimate for γ (a component of $\hat{\boldsymbol{\theta}}$) and $t(n-N)$ is Student's t-distribution with $n-N$ df. The quantities $D(\boldsymbol{X})$ and $E(\boldsymbol{X})$ in (4.13)–(4.16) correspond essentially to variances associated with $\tilde{y}$ and $\hat{y}$, respectively. In (4.14), $E(\boldsymbol{X})$ is expressed as the sum of two terms. These correspond to the two sources of error in $\hat{y}$ cited above.

The distributions (4.15) and (4.16) can be used to obtain either one-sided or two-sided confidence intervals for the various quantities in question. In the present context, one-sided intervals are the most useful, since, for example, it is a low value of ET50 that is problematic. If π is a probability level (such as 95%), let $T(\pi, n-N)$ represent the upper π-level point of the t-distribution with $n-N$ df. Then, (4.15) and (4.16) can be used to show that the following inequalities hold with probability π:

$$(4.17) \quad y^* > [\tilde{y}^{\hat{\gamma}} - \tfrac{1}{2}\sqrt{D(\boldsymbol{X})}\,(V^{\hat{\gamma}} - v^{\hat{\gamma}})\,T(\pi, n-N)]^{1/\hat{\gamma}}$$

$$(4.18) \quad \hat{y} > [\tilde{y}^{\hat{\gamma}} - \tfrac{1}{2}\sqrt{E(\boldsymbol{X})}\,(V^{\hat{\gamma}} - v^{\hat{\gamma}})\,T(\pi, n-N)]^{1/\hat{\gamma}}.$$

The inequality (4.17) gives a lower limit for the true prediction y^*, that is, for the response surface itself. Similarly, (4.18) gives a lower limit for a new observation $\hat{y}$. From (4.14) it follows that

$$E(\boldsymbol{X}) > D(\boldsymbol{X});$$

consequently, the limit defined by (4.18) lies below that defined in (4.17). Again, this relates to the two sources of error in $\hat{y}$.

The right-hand member of (4.18) is the limit $y'(\boldsymbol{X})$ described in the first paragraph of this section. In the particular case where there is only one factor x_1, (4.18) can be written as

$$(4.19) \quad \hat{y} > y'(x_1),$$

an inequality that holds with probability π. If a minimum tolerable response $\hat{y}_{\min}$ is *prescribed*, then the equation

$$(4.20) \quad \hat{y}_{\min} = y'(x_1)$$

can be solved numerically (although not analytically) for x_1. This gives a factor level x_1 which, at confidence level π, will yield a response at least as high as the prescribed $\hat{y}_{\min}$. Determining such a factor level is ultimately the purpose of many toxicology studies.

The formulas for variance and confidence in this section are all approximate, based on approximations commonly used in nonlinear regression problems. In this case, there is one point of departure from the conventional theory. Here, there is an unknown parameter (namely γ) associated with the response y, along with other parameters associated with the factor vector $\boldsymbol{X}$. This point is discussed in mathematical detail in Appendix B, which contains brief proofs of the results presented above.

The equations here, although perhaps mathematically complicated, have been written to allow straighforward programming on a computer. First, the functions F and G_k ($k = 1, \ldots, N$) are defined by (4.2)–(4.8). Second, after the estimate $\hat{\boldsymbol{\theta}}$ is found for a given data set, the matrix $\boldsymbol{H}$, the sum S, and the matrix $\boldsymbol{C}$ can be computed from (4.9)–(4.11). Notice that these quantities are determined completely from the data. Third, given $\hat{\boldsymbol{\theta}}$, S, and $\boldsymbol{C}$, the functions $D(\boldsymbol{X})$ and $E(\boldsymbol{X})$ are defined by (4.13) and (4.14). Notice in particular that $\tilde{y}$ in (4.13) is itself a function of $\boldsymbol{X}$. Finally, lower limits for the true prediction y^* and a new observation $\hat{y}$ at $\boldsymbol{X}$ can be calculated from (4.17) and (4.18). These depend on $\boldsymbol{X}$, just as the familiar confidence curve for an ordinary y-on-x regression line typically lies further from the line as x moves away from the region where data are available. We illustrate all these ideas in a companion paper (Jensen et al. 1986) that gives the complete analysis of a particular data set.

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Appendix A. Derivation of Fig. 1, 2, and 3

The following equivalences are immediate from (2.9):

$$(A.1) \quad z_1^{\beta_1} = 0 \Leftrightarrow z_2^{\beta_2} = g$$

$$(A.2) \quad z_2^{\beta_2} = 0 \Leftrightarrow z_1^{\beta_1} = -g/f.$$

These potentially determine intercepts and asymptotes. For example, if $g > 0$, then (A.1) determines a z_2-intercept ($z_1 = 0$) when $\beta_1 > 0$ and a z_2-asymptote ($z_1 = \infty$) when $\beta_1 < 0$. Also, if (2.9) has an inflection point, then its coordinates $z_i^*(i = 1,2)$ turn out to be given by

$$z_1^{*\beta_1} = \frac{g(1-\beta_1)\beta_2}{f(\beta_1 - \beta_2)} \tag{A.3}$$

$$z_2^{*\beta_2} = \frac{g(1-\beta_2)\beta_1}{(\beta_1 - \beta_2)}. \tag{A.4}$$

Finally, first and second derivatives of z_2 in (2.9) can be written in the forms

$$\frac{dz_2}{dz_1} = \frac{f\beta_1}{\beta_2} z_1^{\beta_1 - 1} z_2^{1-\beta_2} \tag{A.5}$$

$$\frac{d^2 z_2}{dz_1^2} = \frac{1}{z_2}\left(\frac{dz_2}{dz_1}\right)^2 \frac{\beta_1 - \beta_2}{\beta_1}[1 - (z_1^*/z_1)^{\beta_1}]. \tag{A.6}$$

Notice that (A.6) involves z_1^* raised to the power β_1. This quantity can be calculated from (A.3), even if the left side of (A.3) is negative and z_1^* itself fails to exist.

From (A.1)−(A.4), the existence of intercepts, asymptotes, and inflection points depends on the signs of the seven quantities

$$\beta_1, \beta_2, 1-\beta_1, 1-\beta_2, \beta_1 - \beta_2, f, g. \tag{A.7}$$

Furthermore, since z_1 and z_2 both must be positive, the slope (A.5) and acceleration (A.6) also depend on these signs. The first five quantities (A.7) are zero on the various lines drawn in Fig. 1, where each single panel involves only four lines. The last two quantities (f and g) determine a particular panel, (A), (B), or (C). Thus, any region in Fig. 1 is uniquely determined by the quantities (A.7). For example, the region 2a in panel (A) is determined by the condition that all quantities are positive. Region 1a is determined by the sequence of signs

$$+, +, -, \pm, +, +, +,$$

where the $\pm$ indicates that the sign of $1 - \beta_2$ is irrelevant.

Using region 2a (all signs +) as an example, it follows from (A.1) that there is a z_2-intercept; but, from (A.2), there is no z_1-intercept, since $-g/f < 0$. Similarly, from (A.3) and (A.4), there is an inflection point. From (A.5), the curve slopes upward, and from (A.6), it is convex down for z_1 below the inflection point and convex up for z_1 above it. These properties precisely define the curve 2a in Fig. 2. Proofs for all remaining curves are similar.

Appendix B. Proofs of the Variance Formulas

Using notation developed in the paper, the model can be written in the form

$$F(\boldsymbol{X}, \hat{y}, \boldsymbol{\theta}^*) = \sigma\epsilon \tag{B.1}$$

where $\hat{y}$ is an observation, $\boldsymbol{\theta}^*$ is the true (unknown) parameter vector, and ϵ is normal with mean 0 and variance 1. Let $(\boldsymbol{X}_j, y_j), j = 1, \ldots, n$, be a data set with the associated parameter estimate $\hat{\boldsymbol{\theta}}$. Define the following functions of $\boldsymbol{\theta}$:

$$F_j(\boldsymbol{\theta}) = F(\boldsymbol{X}_j, \hat{y}_j, \boldsymbol{\theta}) \tag{B.2}$$

$$f_j(\boldsymbol{\theta}) = \frac{\partial F}{\partial y}(\boldsymbol{X}_j, \hat{y}_j, \boldsymbol{\theta}) \tag{B.3}$$

for $j = 1, \ldots, n$. Then the likelihood function for the data is

$$L = (2\pi)^{-n/2}\sigma^{-n}\prod_{j=1}^{n} f_j \exp\left[-\frac{1}{2}\sigma^{-2}\sum_{j=1}^{n} F_j^2\right]. \tag{B.4}$$

The product Π in (B.4) is a Jacobian required to convert the normal likelihood for ϵ into a likelihood for an observation $\hat{y}$. Let

$$\lambda = -\log L - (n/2)\log(2\pi)$$

represent the negative log likelihood, except for a constant. Then

$$\lambda(\boldsymbol{\theta}) = n\log\sigma + (\sigma^{-2}/2)S(\boldsymbol{\theta}) - R(\boldsymbol{\theta}) \tag{B.5}$$

where

$$S(\boldsymbol{\theta}) = \sum_{j=1}^{n}[F_j(\boldsymbol{\theta})]^2 \tag{B.6}$$

$$R(\boldsymbol{\theta}) = \sum_{j=1}^{n}\log[f_j(\boldsymbol{\theta})]. \tag{B.7}$$

In a conventional least squares problem, the quantities f_j and R do not depend on $\boldsymbol{\theta}$; consequently, the term R in (B.5) is a constant.

From the general theory of likelihood (Kendall and Stuart 1979, p. 59), if $\boldsymbol{C}$ is the covariance matrix of parameter estimates, then the approximation

$$(\boldsymbol{C}^{-1})_{kl} \simeq \frac{\partial^2\lambda}{\partial\theta_k\partial\theta_l}(\hat{\boldsymbol{\theta}}) \tag{B.8}$$

is valid for large sample sizes. From (B.5) it follows that

$$\frac{\partial^2\lambda}{\partial\theta_k\partial\theta_l} = \sigma^{-2}\sum_{j=1}^{n}\frac{\partial F_j}{\partial\theta_k}\frac{\partial F_j}{\partial\theta_l} + \sigma^{-2}\sum_{j=1}^{n}F_j\frac{\partial^2 F_j}{\partial\theta_k\partial\theta_l} - \frac{\partial^2 R}{\partial\theta_k\partial\theta_l}. \tag{B.9}$$

If σ is small and expressions in (B.9) are evaluated at $\hat{\boldsymbol{\theta}}$, then it follows from (B.1) that $F_j = 0(\sigma)$ and $R = 0(1)$, so that the three terms on the right-hand side of (B.9) have orders of magnitude $0(\sigma^{-2})$, $0(\sigma^{-1})$, and $0(1)$, respectively. Keeping only the dominant term of order $0(\sigma^{-2})$ gives, from (B.8) and (B.9),

$$(\boldsymbol{C}^{-1})_{kl} \simeq \sigma^{-2}\sum_{j=1}^{n}\frac{\partial F_j}{\partial\theta_k}(\hat{\boldsymbol{\theta}})\frac{\partial F_j}{\partial\theta_l}(\hat{\boldsymbol{\theta}}). \tag{B.10}$$

Also, from (3.5) and (4.10), an unbiased estimate for σ^2 is

$$\hat{\sigma}^2 = \frac{S}{n-N}. \tag{B.11}$$

Combining (B.10) and (B.11) gives (4.11) and (4.12).

To obtain a lower limit for the unknown true prediction y^*, consider the Taylor expansion

$$F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}) \simeq F(\boldsymbol{X}, y^*, \boldsymbol{\theta}^*) + \sum_{k=1}^{N}\frac{\partial F}{\partial\theta_k}(\boldsymbol{X}, y^*, \boldsymbol{\theta}^*)(\hat{\theta}_k - \theta_k^*). \tag{B.12}$$

Notice, however, that $F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}^*) = 0$ by definition of $\boldsymbol{\theta}^*$. Consequently, since the estimate $\hat{\boldsymbol{\theta}}$ is asymptotically multinormal, it follows that the statistic $F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}})$ is asymptotically normal with mean 0 and variance given approximately by

$$\text{(B.13)}\quad \operatorname{Var}[F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}})] \simeq \sum_{k=1}^{N}\sum_{l=1}^{N} \frac{\partial F}{\partial \theta_k}(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}}) \times \frac{\partial F}{\partial \theta_l}(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}})\, C_{kl}.$$

In passing from (B.12) to (B.13), we use the fact that

$$\text{(B.14)}\quad \frac{\partial F}{\partial \theta_k}(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}^*) \simeq \frac{\partial F}{\partial \theta_k}(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}})$$

to within $0(\sigma)$, since both $y^* - \hat{y}$ and $\boldsymbol{\theta}^* - \hat{\boldsymbol{\theta}}$ are $0(\sigma)$. A short calculation shows that

$$\text{(B.15)}\quad F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}) = F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}) - F(\boldsymbol{X}, \tilde{y}, \hat{\boldsymbol{\theta}}) = \frac{2(y^{*\hat{\gamma}} - \tilde{y}^{\hat{\gamma}})}{V^{\hat{\gamma}} - v^{\hat{\gamma}}},$$

since $F(\boldsymbol{X}, \tilde{y}, \hat{\boldsymbol{\theta}}) = 0$ by definition of $\tilde{y}$. Dividing the normal statistic (B.15) by its estimated standard deviation from (B.13) gives the t-statistic (4.15). Details of this argument follow from linearization and an application of the usual theory for normal variables. The distribution (4.15) implies the lower limit for y^* given in (4.17).

To obtain a lower limit for a new observation $\hat{y}$, consider the expansion

$$\begin{aligned}\text{(B.16)}\quad F(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}}) &= [F(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}}) - F(\boldsymbol{X}, \hat{y}, \boldsymbol{\theta}^*)] + F(\boldsymbol{X}, \hat{y}, \boldsymbol{\theta}^*)\\ &\simeq \sum_{k=1}^{N} \frac{\partial F}{\partial \theta_k}(\boldsymbol{X}, \hat{y}, \boldsymbol{\theta}^*)(\hat{\theta}_k - \theta_k^*) + \sigma\epsilon\\ &\simeq \sum_{k=1}^{N} \frac{\partial F}{\partial \theta_k}(\boldsymbol{X}, y^*, \boldsymbol{\theta}^*)(\hat{\theta}_k - \theta_k^*) + \sigma\epsilon\\ &\simeq F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}}) + \sigma\epsilon.\end{aligned}$$

Line 2 of (B.16) is simply a Taylor expansion combined with (B.1), line 3 is based on the fact that $\hat{y} - y^* = 0(\sigma)$, and line 4 follows from (B.12). Notice that the two variates summed in the final line are independent. The first, $F(\boldsymbol{X}, y^*, \hat{y})$, depends only on past data, and the second, $\sigma\epsilon$, depends only on the random error ϵ in the new data observation $\hat{y}$. Consequently,

$$\text{(B.17)}\quad \operatorname{Var}[F(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}})] \simeq \operatorname{Var}[F(\boldsymbol{X}, y^*, \hat{\boldsymbol{\theta}})] + \sigma^2.$$

Also, a short calculation similar to (B.15) shows that

$$\text{(B.18)}\quad F(\boldsymbol{X}, \hat{y}, \hat{\boldsymbol{\theta}}) = \frac{2(\hat{y}^{\hat{\gamma}} - \tilde{y}^{\hat{\gamma}})}{V^{\hat{\gamma}} - v^{\hat{\gamma}}}.$$

Just as (B.13) and (B.15) lead to the t-statistic (4.15), so (B.17) and (B.18) lead to the t-statistic (4.16), from which the lower limit (4.18) for $\hat{y}$ follows. This completes the proof of all variance formulas and confidence limits in that section.

Assessing Juvenile Salmonid Response to Gas Supersaturation Using a General Multivariate Dose–Response Model

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Based on data compiled from the literature, we investigate the effects of excess total gas pressure (TGP%) on juvenile salmonids (chinook, *Oncorhynchus tshawytscha*; coho, *O. kisutch*; sockeye, *O. nerka*; and steelhead, *Salmo gairdneri*). Our study relates exposure time to 50% mortality (ET50) to TGP%, component levels of dissolved oxygen (O_2%) and nitrogen (N_2%), water depth, temperature, barometric pressure (BP), and fish size. We employ a new device, called a tabular histogram, for exploratory data analysis. We also formulate and test statistical hypotheses by applying the general multivariate dose–response model described in a companion paper (Can. J. Fish. Aquat. Sci. 43: 1684–1693). When modeled in response to TGP%, ET50 follows a typical exponential dose–response relationship. Based on our full data set, two ancillary factors, water depth and fish length, significantly improve the model's ability to explain variations in ET50. Furthermore, based on a smaller subset of the data, either of the factors O_2% or N_2% also improve the model fit significantly. For 50-d exposures, apparently "safe" levels of TGP% range from 103.8 to 114.8%, depending on associated factor levels of water depth and fish size.

À partir de données tirées des publications, les auteurs ont étudié les effets d'une pression gazeuse totale excessive (PGT, %) sur des salmonidés juvéniles (saumons quinnat, *Oncorhynchus tshawytscha*, coho, *O. kisutch*, rouge, *O. nerka* et truite arc-en-ciel, *Salmo gairdneri*). Ils ont mis en relation la durée d'exposition correspondant à une mortalité de 50 % (DE50) avec la PGT, les concentrations en oxygène (O_2, %) et en azote (N_2, %) dissous, la profondeur de l'eau, la température, la pression barométrique (PB) et la taille des poissons. Ils ont fait appel à une nouvelle approche, un histogramme tabulaire, pour l'analyse exploratoire des données et formulé des hypothèses qui ont fait l'objet d'un test statistique de par l'application du modèle général à variables multiples dose–réponse décrit dans une communication connexe (Can. J. Fish. Aquat. Sci. 43 : 1684–1694). La DE50 présentait une relation dose–réponse exponentielle type quand elle était modélisée en réponse à la PGT. L'utilisation de deux facteurs connexes, la profondeur de l'eau et la longueur des poissons, a permis d'accroître de façon appréciable la capacité du modèle à expliquer les variations de DE50 lorsqu'il était appliqué à l'ensemble des données. L'utilisation des teneurs en O_2 ou N_2 permettait aussi d'accroître de façon appréciable l'ajustement du modèle appliqué à un sous-ensemble des données. Dans le cas d'expositions de 50 jours, les valeurs de PGT « sécuritaires » variaient de 103,8 à 114,8 %, tout dépendant de l'importance des facteurs connexes associés, la profondeur de l'eau et la taille des poissons.

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When exposed to water supersaturated with atmospheric gases, fish can develop a problem commonly called "gas bubble disease." The cause is not pathogenic, but physical, resulting from pressure disequilibrium between liquid and gas phases. Hence, "gas bubble trauma" may be a more appropriate phrase (Fidler 1984). At equilibrium the total gas pressure (TGP, millimetres of mercury, where 1 mmHg = 133.3 Pa), defined as the sum of partial pressures of all dissolved gases, equals the barometric pressure (BP, millimetres of mercury). If the differential pressure

$$\Delta P = \text{TGP} - \text{BP} \quad (1.1)$$

is positive, then the water is supersaturated, and gases tend to come out of solution. Aquatic organisms in such an environment can develop gas bubbles or emboli in their organs, tissues, and vascular systems. Such effects were described in the early 1900s (Gorham 1900; Marsh and Gorham 1905). These authors found gas bubbles externally in the fins, along the lateral line, and in the lining of the mouth. Internally they also found bubbles behind the eye, causing exopthalmia or "pop-eye," and within the blood vessels. Vascular bubbles varied in size; they may block vessels, or in extreme cases block blood flow through the heart, resulting in blood stasis and death.

For about half a century subsequent to these early studies, elevated TGP levels ($\Delta P > 0$) were considered to be minor problems, caused either by faulty pumps or water-intake pipes in man-made fish-rearing facilities or by unusual occurrences of photosynthetic activity creating water supersaturated with high levels of oxygen. Then, in the 1960s, serious gas supersaturation events were recognized in the Columbia and Snake

rivers, resulting from the operation of numerous hydroelectric dams. Initially, the problem was assumed to be one of nitrogen supersaturation. By the 1970s, however, it became increasingly clear that gas bubble trauma resulted from supersaturation by all atmospheric gases, rather than just nitrogen alone. Investigations were conducted to determine the lethal and sublethal responses of migrating adult and juvenile salmonids. These, and related studies, are described in reviews by Harvey (1975) and Weitkamp and Katz (1980), and also in the compilation by Fickeisen and Schneider (1976).

Although much effort went into quantifying biological responses to supersaturation, published information in the 1960s and early 1970s suffered from an incomplete understanding of the physical laws governing gas solubilities. For example, various studies have reported on oxygen alone, on nitrogen (more precisely, combined nitrogen and argon) alone, or on TGP alone with no information on the proportions of dissolved gases. In addition, problems arose from difficulties of measurement and analysis and from nonstandard ways of reporting TGP.

Measurement of TGP prior to the early 1970s required measuring each component gas in solution. While this was not a problem for oxygen, nitrogen could be measured only by the tedious manometric method of Van Slyke (1927) or by laboratory use of a gas chromatograph (D'Aoust and Clark 1980). In 1971 a very simple membrane-diffusion method was developed for measuring TGP. The instrument, the "Weiss saturometer" (Colt 1983) and several later models called "tensionometers and gasometers" (D'Aoust et al. 1976; Bouck 1982), consists of gas-permeable silicone rubber tubing connected to a pressure-sensing device. The tubing, although impermeable to liquids, allows passage of gases and water vapour until pressure equilibrium between the inside and the outside of the tubing is achieved. The result is a direct measure of ΔP, the driving force required for bubble growth. Harvey (1975) and Colt (1983) gave excellent discussions on the principal factors and physical laws that govern dissolved gases in water. In addition, Colt (1983) proposed standards for computation and reporting of gas supersaturation in freshwater and seawater. These landmark papers make clear the necessity of obtaining measurements not only of TGP, but also BP, oxygen (or the oxygen/nitrogen ratio), water temperature, salinity (if applicable), and water depth. Only by considering all of these factors can one understand fully whether or not fish may be subjected to gas disequilibrium and develop gas bubble trauma.

Total gas pressure traditionally has been reported as a percentage of complete saturation, based on the equation

$$(1.2)\quad \mathrm{TGP\%} = 100\,(\mathrm{BP} + \Delta P)/\mathrm{BP}$$

where ΔP either is measured directly with a tensionometer or is computed indirectly by adding the measured partial pressures of component gases, including water vapour. In (1.2), BP typically is much larger than ΔP, so that moderate changes in BP result in relatively small alterations in TGP%. For example, suppose that ΔP = 76 mmHg, and consider the difference in TGP% between two sites at different altitudes (AL, metres), one at sea level (20°C) and the other at 1500 m. Then BP, 760 mmHg at sea level, is reduced to 638 mmHg using the equation (Colt 1980)

$$(1.3)\quad \log(\mathrm{BP}) = 2.8808 - (\mathrm{AL}/19748.2).$$

Consequently, TGP%, which is 110% at sea level, is increased to 112% at 1500 m.

Another pressure component, hydrostatic pressure, may have a more dramatic effect on TGP%. In (1.2), both BP and ΔP are normally taken to be pressures at the water surface. Indeed, the membrane-diffusion method measures the surface ΔP, regardless of the depth of the probe, since the membrane essentially blocks water pressure from the sensing device. However, bubble growth is dependent on differential pressure at a given depth. The total pressure (TP, millimetres of mercury) at depth D (metres) is (Colt 1983)

$$(1.4)\quad \mathrm{TP} = \mathrm{BP} + 73.56\rho D$$

where ρ (grams per cubic centimetre) is the water density and 73.56 is the conversion factor from metric pressure to millimetres of mercury. (Essentially, a column of about 74 mmHg will balance a 1-m column of water.) Assuming uniform mixing of water and gas from the surface to depth D, this corrected TP should be used instead of BP in the denominator of (1.2). Thus, TGP% becomes a function of D:

$$(1.5)\quad \mathrm{TGP\%}(D) = 100\,(\mathrm{BP} + \Delta P)/(\mathrm{BP} + 73.56\rho D).$$

At 20°C, ρ is 0.9982 g/cm^3 for freshwater. Therefore, at a BP of 760 mmHg, a depth D of 1 m, and a measured surface ΔP of 76 mmHg, TGP% is 100.3%. In other words, in a uniform water column with TGP% = 110% at the surface, gas bubble growth cannot occur at a depth of 1 m. In general, each metre of water depth compensates approximately for a 10% increase in TGP%.

Two important considerations follow from the knowledge that hydrostatic pressure can have a pronounced effect on bubble growth. First, many investigators have tested fish in tanks or water columns 0.6 m or more in depth, without noting at what depth the fish resided. Consequently, TGP% levels may be reported with large errors related to the depths of enclosures employed. Second, salmonids may be able to detect and avoid lethal TGP% levels by swimming to a greater depth. Weitkamp and Katz (1980) suggested that insufficient evidence exists to draw useful conclusions on this issue. Recent data (M. Shrimpton, University of British Columbia, Vancouver, B.C., pers. comm.) affirm the fact that young salmon indeed move to greater depths in the water column at TGP levels greater than 100% saturation. However, the question of whether these fish detect excess total gas pressure or respond to the symptoms of gas bubble trauma remains unanswered.

The main objective for much experimentation since the 1960s has been to determine safe TGP% levels for salmonids migrating through the Columbia and Snake rivers and, more recently, for salmonids held in shallow hatchery incubation and rearing environments. The sources of high TGP% in the former case have been numerous dams, creating TGP% levels of 120% or greater by entraining air in the plunge of water over spillways. In hatcheries, TGP% levels from 102 to 110% may occur naturally or be produced by heating or mechanical entrainment of air (e.g. from the use of aspirators or faulty pumps).

A recent examination of the literature (Alderdice and Jensen 1985) has suggested a division of mortality responses into two categories, chronic and acute, resulting from extravascular and intravascular bubble growth, respectively. Thus, the relationship between mortality and TGP% may exhibit two phases, each associated with different etiologies and rate processes. Indeed, such a compound relationship was described by Bengtsson (1974) for the response of the minnow *Phoxinus*

TABLE 1. Source authors and record frequencies for data sets 1 and 2.

Source	No. of records	
	Set 1	Set 2
Meekin and Turner 1974	15	15
Dawley and Ebel 1975	4	4
Nebeker et al. 1979	121	35
Rucker 1975b	12	12
Blahm et al. 1975	1	1
Stroud and Nebeker 1976	1	0
Nebeker et al. 1976	14	14
Nebeker and Brett 1976	3	0
Total	171	81

phoxinus to zinc. A boundary between chronic and acute gas bubble trauma appears to lie in the TGP% range between 108 and 110%. Practical experience in the two major problem situations, below dams and within hatcheries, seems to bear out this distinction. The levels of TGP% encountered below dams often are acutely lethal, causing bubble growth in the blood circulatory system and other tissues. In hatcheries the TGP% levels, being much lower, are not rapidly lethal but can cause chronic low-level mortality in salmonid larvae and juveniles (Harvey and Cooper 1962; Wedemeyer et al. 1976; Wright and McLean 1985).

Various agencies have recommended safe levels of TGP%, below which aquatic species are not expected to be adversely affected, ranging from 105% in Oregon to 110% in Washington and Idaho (Weitkamp and Katz 1980). Ebel et al. (1979), in reviewing the most recent Environmental Protection Agency criteria, indicated that dissolved gas criteria could be established at either 110, 115, or 120%. It is not surprising that such a range in dissolved gas criteria can exist, given the variety of test conditions used in examining response to TGP.

The difficulties in measurement and the problems of nonstandard reporting may account for some of this variability. However, the main difficulty in interpreting biological response to gas supersaturation is the fact that ancillary factors also appear to have strong influences. We have discussed how two ancillary factors, barometric pressure and water depth, influence the computed TGP%. The modifying influences of high temperature (Ebel et al. 1971; Nebeker et al. 1979) and oxygen/nitrogen ratios (Rucker 1975a; Nebeker and Brett 1976; Nebeker et al. 1979) have also been demonstrated. Possible influences of species, life stage, and fish size were discussed by Weitkamp and Katz (1980). Hence, it seems reasonable to establish dissolved gas criteria by modeling lethal responses not only to TGP%, but also to ancillary factors when such information is available.

The objective of this paper is to model exposure times necessary to cause 50% mortality (ET50, hours) in a salmonid population exposed to levels of TGP% higher than 100% (i.e. dissolved gas supersaturation). In a separate report (Jensen et al. 1985), literature data have been compiled to obtain, where possible, information on species, life stage, fish size (length and weight), water temperature, barometric pressure, water depth, percentage mortality, exposure time, and the proportions of dissolved gases. The present paper investigates these data, first, with a simple exploratory device defined as a "tabular histogram." Then the data are modeled in stepwise fashion using a general multivariate dose–response model described in the companion paper by Schnute and Jensen (1986). Finally, we employ the models that best explain ET50 to estimate safe levels of gas supersaturation.

Exploratory Data Analysis

The literature search for quantitative data on salmonid mortality response to excess gas pressure yielded 621 records from 23 publications. These and two subsets of the data have been compiled elsewhere (Jensen et al. 1985). Our analysis here is confined to the two data subsets, which we refer to as data sets 1 and 2 (Table 1). Each contains information on ET50 among juvenile salmonids exposed to excess gas pressure, together with information on various ancillary variables. Data set 1, comprising 171 records, includes the ancillary variables water depth, temperature, fish length, barometric pressure, and fish species. Data set 2 consists of 81 records from data set 1 for which ancillary information on oxygen and nitrogen levels is also available.

Before describing the data sets in detail, it is necessary to clarify the meanings of four quantities related to gas pressure. The first, TGP%, has already been discussed. Within both data sets, this is reported as the *surface* gas saturation level, given by (1.2) or (1.5) with $D = 0$. Any depth effect is considered separately by admitting water depth as an ancillary variable. The second quantity, $O_2\%$, is the percentage saturation of oxygen at the surface in relation to the equlibrium level of dissolved oxygen associated with normal air. Similarly, the third quantity, $N_2\%$, is the percentage saturation of combined nitrogen and argon in relation to equilibrium with normal air. Due to the relative amounts of oxygen and nitrogen in normal air, the quantities TGP%, $O_2\%$, and $N_2\%$ are connected approximately by the relationship

$$(2.1) \quad \text{TGP\%} = (0.21)\, O_2\% + (0.79)\, N_2\%.$$

A more precise version of (2.1) is well known in the literature (e.g. Colt 1983, table 1); however, (2.1) reflects the precision available in the literature data used here. Finally, the fourth quantity, the oxygen/nitrogen ratio (ONR), is defined by

$$(2.2) \quad \text{ONR} = O_2\%/N_2\%.$$

Notice that the four quantities TGP%, $O_2\%$, $N_2\%$, and ONR are constrained by the two equations (2.1) and (2.2). Thus, any two of these quantities determine the other two.

Tables 2 and 3 summarize the information in data sets 1 and 2, respectively. They include the mean, minimum, and maximum for each variable. Furthermore, to represent the distribution of each variable, the tables include a simple graphic device that we call a "tabular histogram." We use this name because the device contains the essential information in a histogram and yet fits easily in a table. To be more precise, Tables 2 and 3 include a 10-cell one-dimensional tabular histogram for each variable, where the cells are defined by dividing the interval between the variable's minimum and maximum value into 10 equal intervals. A histogram consists of the symbols "1" through "9", "*", and ".", where the symbol for each cell represents the cell frequency on a scale from 1 to 9. In particular, the cell with the largest number of observations (the "maximum cell count" in Tables 2 and 3) is always coded "9". To enhance the histogram's visual impact, cells with only 0 or 1 observation are considered special cases and designated with a "." or "*", respectively.

TABLE 2. Descriptive statistics for data set 1 (171 records), including means, minima, maxima, and tabular histograms with maximum cell counts. The histograms represent cell counts on a scale of "1" to "9". The symbols "." and "*" represent the particular instances of 0 and 1 records, respectively.

Variable and code description	Mean	Minimum	Maximum	Histogram	Max. cell count
y ET50 (h)	139.3	1.5	1182.0	932111...*	109
ln y ln (ET 50)	4.02	0.41	7.07	3322496751	39
x_1 TGP % (%)	122.6	111.0	141.4	1489.23.22	55
x_2 Depth (m)	0.54	0.14	1.00	21...9...*	144
x_3 Temperature (°C)	13.1	8.0	20.0	3139252.31	60
x_4 Fish length (mm)	119.8	45.0	209.0	31898392*3	36
x_5 BP (mmHg)	755.6	736.0	761.6	3.....3992	64
Species[a]	—	1	4	4..3..5..9	81

[a]1 = chinook, 2 = coho, 3 = sockeye, 4 = steelhead.

TABLE 3. Descriptive statistics for data set 2 (81 records), including means, minima, maxima, and tabular histograms with maximum cell counts. The histograms represent cell counts on a scale of "1" to "9". The symbols "." and "*" represent the particular instances of 0 and 1 records, respectively.

Variable and code description	Mean	Minimum	Maximum	Histogram	Max. cell count
y ET 50 (h)	79.8	1.5	1182.0	91*1**...*	67
ln y ln (ET50)	3.06	0.41	7.07	8844496221	16
x_1 TGP% (%)	126.2	111.0	141.4	3179.67.45	18
x_2 Depth (m)	0.47	0.14	1.00	41...9...*	54
x_3 Temperature (°C)	12.8	8.3	16.0	*..49.3.33	35
x_4 Fish length (mm)	112.5	45.0	150.0	121612*1*9	35
x_5 BP (mmHg)	753.8	736.0	760.0	3.....3.9	52
x_6 O_2% (%)	117.2	40.5	307.9	239511****	37
x_7 N_2% (%)	128.3	97.3	163.9	1*29843111	26
x_8 ONR	0.94	0.25	3.16	14911.*.**	47
Species[a]	—	1	4	4..3..4..9	38

[a]1 = chinook, 2 = coho, 3 = sockeye, 4 = steelhead.

In data set 1 (Table 2) notice that the response variable y (ET50) ranges from 1.5 to 1182 h with most of the data skewed towards low values of y (i.e. rapidly lethal responses). The log-transformed responses, in comparison, are more uniformly distributed. The major explanator, TGP% (x_1), ranges from 111.0 to 141.4% with little information at low levels. In fact, there are only six records with TGP% below 115%. Most tests (144 records) were performed with a water depth (x_2) of 0.6 m. A reasonable range of depths is available, however, between 0.1 and 0.3 m, although this is not evident in the tabular histogram. Water temperatures (x_3) and fish lengths (x_4) are fairly evenly distributed over their ranges. Levels of BP (x_5) are skewed towards the maximum value. The narrow range of BP levels available in the literature data makes it unlikely that a significant BP effect can be proved from these data. Four salmonid species are represented, including 28 chinook (*Oncorhynchus tshawytscha*), 23 coho (*O. kisutch*), 39 sockeye (*O. nerka*) and 81 steelhead (*Salmo gairdneri*) records.

In data set 2 (Table 3) the distribution of y (ET50) differs little from the distribution in data set 1,the range remaining the same with most of the data skewed toward small values of y. Similarly, the ranges of x_1 (TGP%), x_2 (depth), and x_5 (BP) are essentially unchanged, with points removed primarily from the middle of each range. By contrast, x_3 (temperature) and x_4 (fish length) have reduced ranges, due primarily to the loss of points with high values of x_3 and x_4. Data set 2 also includes the three variables O_2% (x_6), N_2% (x_7), and ONR (x_8) discussed earlier. Most oxygen levels lie between 80 and 140%, and nitrogen levels are concentrated between 120 and 140%. Although ONR ranges from 0.25 to 3.16, roughly 80% of the records in this data set have ONR values close to 1.0. The species in data set 2 appear in nearly the same proportions as in data set 1, as evidenced by the fact that the two tabular histograms are almost identical. Explicitly, data set 2 includes 16 chinook, 13 coho, 14 sockeye, and 38 steelhead records.

To explore possible interactions between the main explanator x_1 (TGP%) and the ancillary variables (x_2–x_8), Tables 4 and 5 present a series of two-dimensional (9 × 9) tabular histograms for data sets 1 and 2, respectively. These histograms represent record frequencies and scaled response plots of y (ET50) and ln y for each combination of x_1 plus one of the variables x_2–x_8. The frequency histograms follow the notation (using "1" through "9", "*", and ".") described earlier for one-dimensional histograms. A cell in a two-dimensional histogram is a rectangle of values for x_1 and x_i, with $i = 2, \ldots, 8$. On the horizontal axis, values x_1 are divided into nine equal intervals between the minimum and maximum for x_1. Similarly, on the vertical axis, values of x_i ($i > 1$) are divided into nine equal intervals between the minimum and maximum for x_i. This gives a total of 81 (9 × 9) rectangular cells for the histogram. The character shown in any cell position represents the number of data points available within that cell. One can inspect the